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Seasonal differentiation of hypogeous fungi in rodent diet in selected forest reserves of Central Poland

Sezonowe zróżnicowanie grzybów podziemnych w diecie gryzoni w wybranych rezerwatach leśnych Polski Środkowej

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1. INTRODUCTION

Although observations of mycophagy (or fungivory) have been known from ancient times, studies of the phenomenon date back to the late 19th century, when naturalists have started observations of mammal mycophagy, spore dispersion of mycorrhizal fungi and its influence on forest life (Maser et al. 1978b; Maser, Maser 1988a; Luoma et al. 2003). Mammalian mycophagy on mycorrhizal hypogeous fungi and its significance for forest ecosystems is a subject of many documented studies throughout Europe, the Americas and Australia (Maser et al. 1978a; Claridge et al. 1999). Due to the variety of relationships between the organisms involved, this topic might be analysed from many points of view, from forest ecology to animal physiology (Johnson 1996; Claridge, Lindermayer 1998). First studies on this matter were conducted in Great Britain and subsequently in the United States. On this basis scientists formulated a hypothesis that fungal spores pass through the animal's digestive tract unchanged. At the beginning of the 20th century, there have already been conducted studies on rodent behaviour. It has been speculated that mushrooms, especially hypogeous fungi, are an important element in rodent diet (Luoma et al. 2003).

In Poland research on mammalian mycophagy on hypogeous fungi has been popularised during participation of Polish scientists in the International Biological Programme (IBP). The Programme promoting international scientific cooperation, had a enormous impact on development and stimulation of ecological research in Poland. IBP was carried since 1964 to 1973, but some studies were conducted as early as 1959. The studies conducted within the IBP were focused on productivity of terrestrial communities in particular and on relationships and interactions between organisms (Andrzejewska 2004). A part of IBP were the zoological studies of Drożdż (1966, 1968), who examined food habits of the bank voles *Myodes* (*Clethrionomys*) *glareolus* and yellow-necked mice *Apodemus flavicollis* in Ojców National Park. Drożdż found fungal spores in stomachs of bank voles, which led him to the hypothesis that fungi can be an important component of animal diet. Taxonomical studies of the gathered material conducted in cooperation with mycologists led to identification of several species of hypogeous fungi, *inter alia* the spores of the valued *Tuber aestivum*, this way confirming its occurrence in Poland.

In later years, mammalian mycophagy was studied in regards to wild boars *Sus scrofa* in Kurpiowska Forest by Genov (1981, 1982), who found spores of *Elaphomyces* both in stomachs and faeces of studied animals. Ławrynowicz et al. (2006) also searched for hypogeous fungi in places rooted by wild boars in Kurpiowska Forest. They confirmed that *Elaphomyces* occurs in rooted places and brought forth the hypothesis, that wild boars while actively searching for hypogeous fungi enable the development of sporocarps by rooting the ground and allowing water access to the soil where the mycelium is present. The study has led to the conclusion, that relationships between animals and fungi exceed trophy and dispersion aspects, and are far more important for the ecosystem as a whole.

By feeding on hypogeous fruit bodies animals take part in spore dispersion in one or more of the following ways (Cork, Kenagy 1989; Johnson 1996; Trappe, Claridge 2005; Trappe et al. 2009):

1) by releasing spores into the air through digging up and opening the sporocarp;
2) by consumption and spreading spores in their faeces;
3) by carrying the spores on the body surface after encountering a over-ripped fruit body.

Many animals can be described as mycophagous and use fungi as a food source to various degrees. Describing these degrees, Claridge, Trappe (2005), distinguished four types of mycophages:

1) obligatory mycophages - feeding wholly or in majority on fungi;
2) preferable mycophages - actively searching out fruit bodies and only seasonally feeding on a different food source;
3) opportunistic mycophages - feeding on mushrooms when this food source is available;
4) accidental mycophages - feeding on fungi while searching for a different food source.

Among mammals most common are opportunistic or accidental mycophagy with rare cases of preferential mycophagy. Most species of mycophagous mammals originate from the families Sciuridae (squirrels), Cricetidae (hamsters and voles) and Geomyidae (gophers). Larger mammalian mycophages originate from families Cervidae (deers) and Suidae (boars and pigs). Further examples are the families Zapodinae (jumping mice), Phascolomidae (wambats), Macropodidae (kangaroos and walabies)

and Leporidae (hares) (Whitaker 1962; Fogel, Trappe 1978; Maser et al. 1978a; Maser et al. 1988; Taylor 1992; Taylor et al. 2009). Fungal spores are also found in stomach contents and faeces of predatory mammals, due to their consumption of mycophagous prey. This phenomenon is called a secondary mycophagy, and can also contribute do spore dispersion. Secondary mycophages can be found amongst small mammals like the Soricidae (shrews) (Whitaker 1962; Fogel, Trappe 1978; Maser et al. 1978a; Rhodes 1986; Kataržytė, Kutorga 2011), and larger predators like bobcats (Nussbaum Maser 1975). Mycophagy was also observed among primates (Harrison 1984; Hanson et al. 2003; Hilario, Ferrari 2011; Sawada et al. 2014).

The aim of this study is to examine the significance of hypogeous fungi in diet of rodents in the forest ecosystem of Central Poland. The study will verify the hypothesis that hypogeous fungi are an important component of rodent diet and that mycophagy plays a significant role in the forest ecosystem. For this purpose, the Author examined the occurrence of spores in faecal samples from two species of rodents: bank vole *Myodes glareolus* and yellow-necked mouse *Apodemus flavicollis*. Both species are widely spread in the Palaearctic and abundant in forest ecosystems and are reported as preferential or opportunistic mycophages (Kataržytė, Kutorga 2011; Schickmann et al. 2012). In particular the following issues were of a special concern:

1) the diversity of fungal genera in faecal samples.
2) difference in spore occurrence in samples obtained in three seasons: spring, summer and autumn;
3) differences in spore occurrence in relation to study area, animal species and animal's sex and age;

This is the first study of this kind conducted in central Poland and is based on original field research and microscope analysis of samples gathered in the field.

2. STUDY AREA

2.1. Overall description

The study presented in this dissertation was carried out by live trapping two common rodent species: bank vole *M. glareolus* and yellow-necked mouse *A. flavicollis*. Two study plots were situated in two nature reserves: Spała (51°31'37" N 20°08'42" E) and Konewka (51°04'08" N 20°09'26" E), located in Pilica Forest, in Łódzkie Voivodship in central Poland. The study was conducted between July 2013 and May 2015.

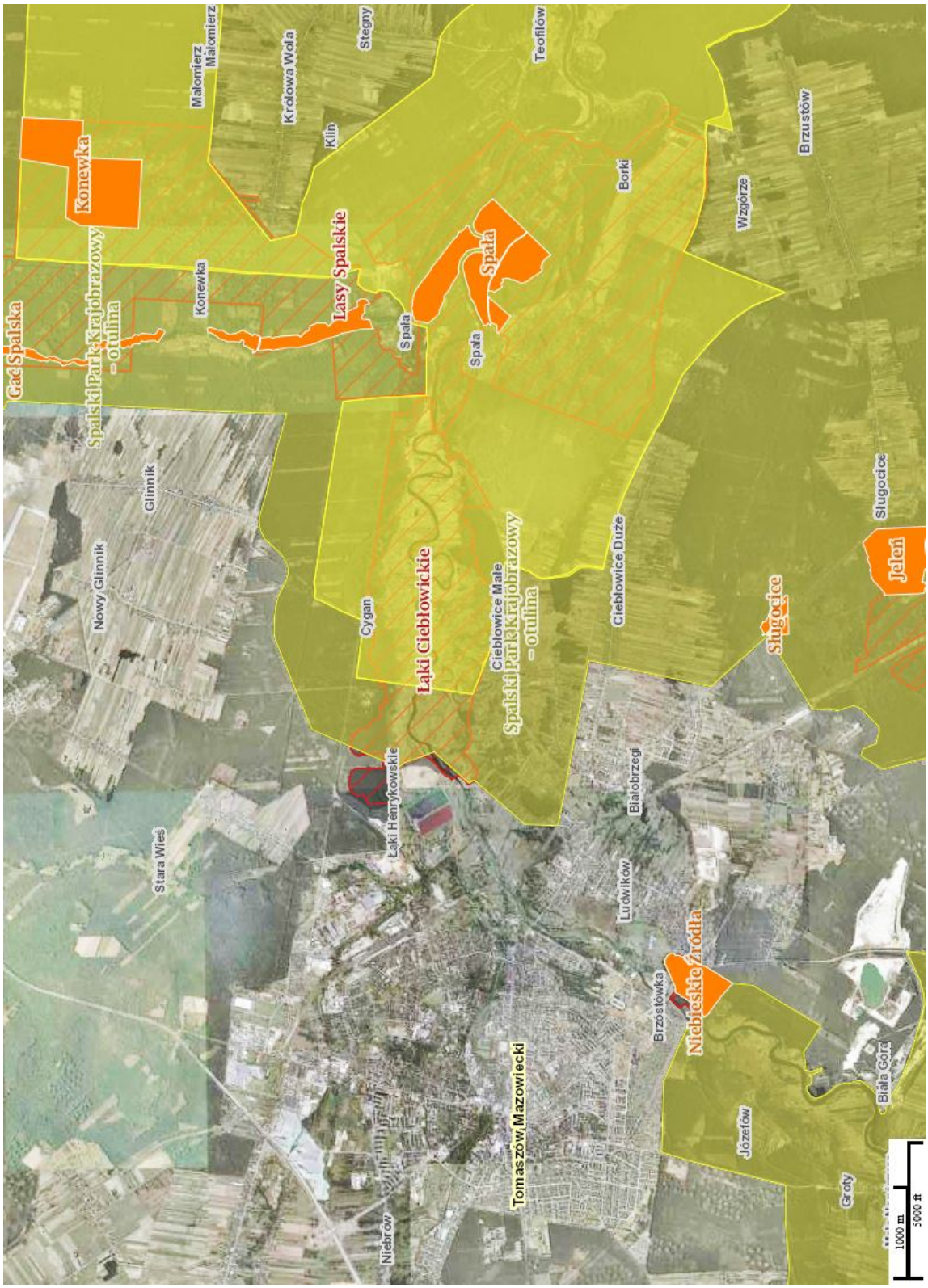
Administratively, the study area is located in the Spała Forest Inspectorate, subordinate to the Regional Forestry Director in Łódź. Geographicaly, it is located in the Piotrkowska Plain, South Masovian Uplands in the central Poland Lowlands, a part of the North European Plain (Kondracki 1978). The study area is located within protected areas: the Spalski Landscape Park, a NATURA 2000 Refuge.

Spalski Landscape Park (SLP) (see map 1) is a part of the "Spalsko-Rogowskie Forests" Promotional Complex. The Park was created in 1995 (Dz. Urz. Woj. Piotrkowskiego 1995.15.113). Its area is 12 875 ha with 57,4% covered by forests and 35,6% by grasslands and rural areas. Water bodies occupy 2,7% of the area. The buffer zone is 23 192 ha, with 63,2% covered by forests, 32,3% by rural areas, and the remaining 4,5% are invested areas and water bodies (Burzyński et al. 1998). South part of the SLP is a NATURA 2000 Refuge PLH100003 "Lasy Spalskie". Its area is 2016,4 ha. Through the SLP and the NATURA Refuge runs the Pilica river, which also runs through the centre of the Spała nature reserve (Kurowski et al. 2013). The river is a unregulated, flowing in its natural riverbed with multiple oxbows, islands, and shoals. Its banks are sandy and accessible. The mean annual flow of Pilica in Tomaszów Mazowiecki is ca. 25 m^3/s. The second major water body near the study plots is the river Gać, a left bank tributary of Pilica, flowing through the forests of Konewka and disgorging itself into Pilica in Spała (Baliński 1996).

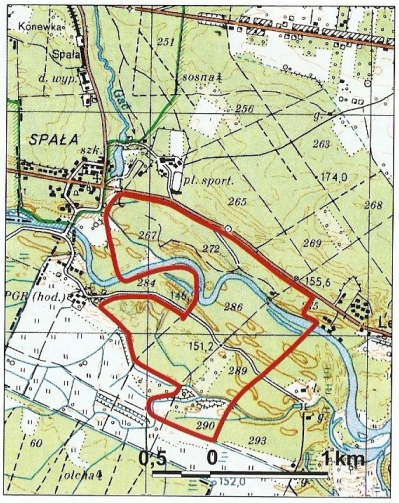
The Spała nature reserve (hereafter referred to as "Spała") was established in 1958 (M.P. 1958.81.467; Dz. Urz. Woj. Łódzkiego 2001.206.2976; Dz. Urz. Woj. Łódzkiego 2014.124) and its area is 106,75 ha. The vegetation of the reserve consists of a subcontinental oak-hornbeam forest *Tilio-Carpinetum*, ash-alder riparian forest

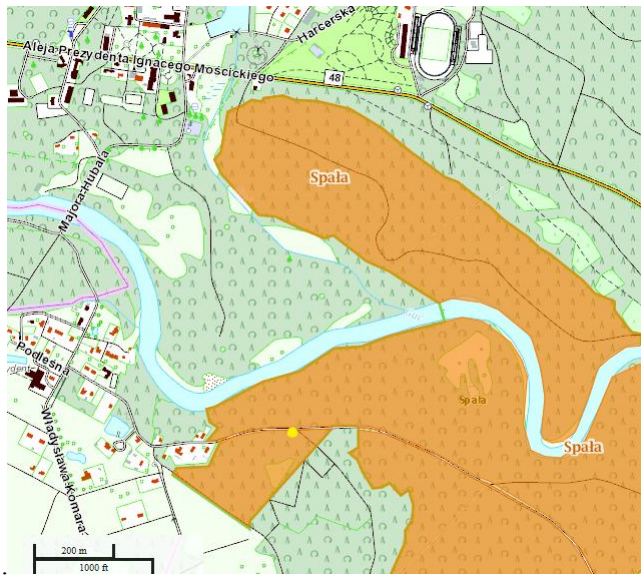
Fraxino-Alnetum and, in a smaller extend, willow-poplar riparian forest *Salici-Populetum*. Within the tree stand, one can find 250-year old oaks *Quercus* spp. and Scots pines *Pinus sylvestris* ranging from 170 to 200 years old. The oldest oaks are up to 30 m high with trunk up to 5 meters in perimeter. Apart from oak and Scots pine in Pilica Forest there are: linden *Tilia cordata*, maple *Acer platanoides*, sycamore *A. pseudoplatanus*, hornbeam *Carpinus betulus*, beech *Fagus sylvatica*, fir *Abies alba* and spruce *Picea abies*. Many old trees, low density of the stand and high number of fallen and dead trees give the reserve the characteristics of a primeval forest (Baliński 1996). In the lower forest layers occur: *Corydalis solida, Anemone nemorosa, Anemone ranunculoides, Asarum europaeum, Pulmonaria obscura,* invasive *Cardamine impatiens, Jacobaea paludosa, Ficaria verna, Gagea lutea, Stellaria nemorum, S. holostea, Melandrium rubrum*, *Hierochloë odorata* (Wnuk, Olaczek 1999; Olaczek 2013) and *Hepatica nobilis* (Kiedrzyński 2008). The study plot in Spała (maps 2, 3) was set on the south bank of Pilica river, about 1 km from human settlements, and along the dirt road through the forest from Spała to Inowłódz. 20 live traps were placed in a less dense plot on the right side of the road, in a forest consisting mainly of oak, birch and hornbeam and with a clearing in the center. 10 traps were placed on the left side in a dense Scots pine forest tree nursery.

The Konewka nature reserve (hereafter referred to as "Konewka") is located 1 km north-east from Konewka village. The reserve occupies 99,31 ha and was created in 1987 (M. P. 1978.33.126; Dz. Urz. Woj. Łódzkiego 2001.206. 2976; Dz. Urz. Woj. Łódzkiego 2010.194.1566). It is a 170-270 year old oak forest with Scots pine. It is classified as a thermophilic oak forest *Potentillo albae-Quercetum* and subcontinental oak-hornbeam forest *Tilio-Carpinetum*. The former typically has a low density and a poorly developed understory (Baliński 1996). Among herbs appear: *Potentilla alba, Ranunculus polyanthemos, Serratula tinctoria, Campanula persicifolia, Hypericum montanum* and *Vaccinium myrtillus* (Wnuk and Olaczek 1999; Olaczek 2013), *Aquilegia vulgaris, Convallaria majalis, Melittis melissophyllum, Lilium martagon, Frangula alnus, Primula veris, Viburnum opulus, Pseudoscleropodium purum* and *Carex montana* (Kiedrzyński 2008). The study plot was located at the outskirts of Konewka (maps 4, 5), along the fence surrounding the Bunker Museum in Konewka village, with 20 live traps placed in a dense tree stand, mostly oak and birch.

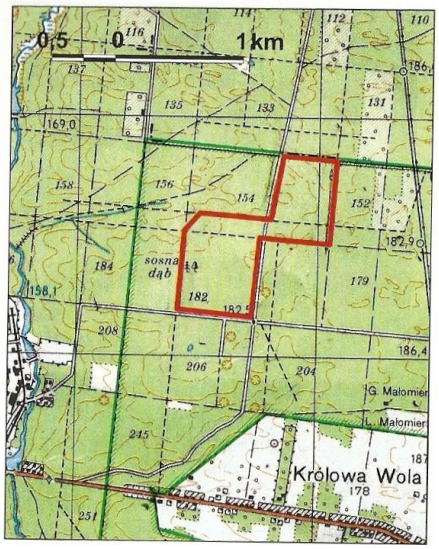


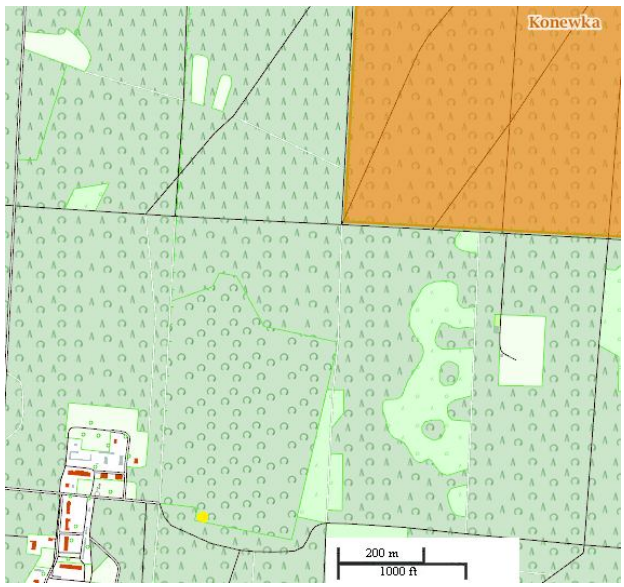
Map 1. Map of the study area, with Spalski Landscape Park (yellow full colour), nature reserves (orange full colour) and NATURA 2000 areas (red shaded colour). Map sampled from Geoserwis GDOŚ website (geoserwis.gdos.gov.pl).

2. 

3. 

Map 2. Map of the Spała reserve (red outline) (Rąkowski 2006).
Map 3. Map of the Spała reserve (orange full colour) with the study plot (yellow dot) (sampled from Geoserwis GDOŚ website geoserwis.gdos.gov.pl)

4. 

5. 

Map 4. Map of Konewka reserve (red outline) (Rąkowski 2006).
Map 5. Map presenting Konewka reserve (orange full colour) and the study plot (yellow dot) (sampled from Geoserwis GDOŚ website geoserwis.gdos.gov.pl).

2.2. Mycological background

Hypogeous fungi are prominent in most forest ecosystems (Molina et al. 2001). This ecological group is extremely difficult to find and study. Their mycelium can be widely spread underground in ectomycorrhizal association with trees. Fruit bodies are produced only in specific locations where biotic and abiotic conditions are met. Furthermore, the fungi can produce fruit bodies through decades, but they do not

produce them annually and some species only fruit every couple of years (Ławrynowicz 1988). The mycological background for the study area is based on the documentation and literature provided by mycological, floristic, and vegetation studies in the Landscape Park and both nature reserves.

The most frequently mentioned among those found in Spała and Konewka were the ascomycetes: *Elaphomyces asperulus*, *E. muricatus*, *E. granulatus*, *Hydnotrya tulasnei* and *Genea hispidula* (Ławrynowicz 1973, 1979, 1984, 1989, 1990), and in Konewka *Pachyphloeus melanoxanthus*, a species associated with *Potentillo albae-Quercetum* community (Ławrynowicz, Grzesiak 2009). Worth mentioning are also species that can potentially occur in the *Tilio-Carpinetum* community: *Choiromyces venosus*, two species of *Tuber, T. puberulum* and *T. borchii*, and glomeromycetes *Endogone/Glomus macrocarpa* (Ławrynowicz 1973, 1979, 1984).

In comparison to ascomycetes information on hypogeous basidiomycetes of the selected area is scarce. A preliminary checklist of Polish Basidiomycota is currently in preparation (Mleczko, Ławrynowicz unpublished data). Considering aforementioned unpublished data and the Checklist of Polish Larger Basidiomycetes (Wojewoda 2003) it is possible that fungi from genera *Rhizopogon* (*R. nigriscens, R. obtextus, R. roseolus*), *Hymenogaster* (*H. tener*), and *Melanogaster* (*M. ambiguous, M. broomeianus, M. variegatus*) also occur in the studied reserves.

2.3. Weather conditions during sample gathering

The climate of the area is more humid and colder than on the surrounding lowlands, due to a significant elevation of the South Masovian Uplands (Kondracki 1978). The annual sum of rainfall in the study area is 644 mm and the mean annual temperature is 7,6°C. The important factor responsible for the mild climate of this terrain are the large forest areas, mostly pine forests on permeable grounds (Baliński 1996).

The weather conditions during the trappings represent three studied seasons – spring, summer and autumn. The conditions taken into consideration were average, maximum and minimum temperature, relative humidity, rainfall, average and maximum wind speed, fog and storms. Data on weather condition were collected from Tutiempo web-base (en.tutiempo.net) for the nearest surveyed location – Sulejów (Table 1).

During the two years of the study, the weather conditions in given seasons were quite stable. In May, the average temperature ranged from 13,2 to 12,6 ^{o}C, the maximum temperature ranged from 18,9 to 17,9 ^{o}C and the minimum temperature 7,8-7,5 ^{o}C. Respectively, the temperatures in July were: 19,3 – 20,0 ^{o}C, 25,7 – 26,3 ^{o}C and 12,5 – 14,4 ^{o}C, and in October: 9,8 – 9,5 ^{o}C, 15,1 – 15,0 , 4,6 – 5,2 ^{o}C.

Table 1. Mean weather conditions: temperature (in ^{o}C) – average (T) maximum (TM) and minimum (Tm), relative humidity (H – in %), rainfall (PP in mm), average and maximum wind speed (V and VM in km/h), number of rainy days (RA), number of days with storms (TS) and number of days with fog (FG) (data collected from en.tutiempo.net for Sulejów).

Date	T	TM	Tm	H	PP	V	VM	RA	TS	FG
07.2013	19,3	25,7	12,5	69,7	1,1	10,5	19,2	9	3	0
10.2013	9,8	15,1	4,6	82,1	0,3	10,8	18,5	11	0	13
05.2014	13,2	18,9	7,8	75,9	5,2	11,8	21,0	19	7	5
07.2014	20,0	26,3	14,4	74,9	4,5	9,4	18,3	16	14	1
10.2014	9,5	15,0	5,2	86,8	1,2	89,5	15,9	10	0	13
05.2015	12,6	17,9	7,5	71,0	1,2	10,4	17,2	6	0	1

Between the years, the seasons varied in rainfall and storms, with seasons in 2014 having more rainy and stormy days (May – 19 and 7 respectively, July – 16 and 14, October – 10 rainy days and no storms) than in 2013 (July – 9 and 3, October 11 rainy days and no storms) and 2015 (May – 6 rainy days and no storms).

2.4. Soil analysis

The geology of the SLP is diverse with Mesozoic structures next to Pleistocene accumulations and late Pleistocene and Holocene erosive forms. The outer layers of the Pilica river valley consist of middle-Jurassic lime-ferric sandstone in Inowłódz and upper-Jurassic limestone west of Inowłódz. The surface layer is covered by forms of glacier accumulation, constituting the highest hills. The landscape is flat with local elevations, mostly reaching 210-220 m a.s.l. The dominant feature of the landscape is a sandy outwash plain with large forest patches. The main watercourse of this region is river Pilica, with an artificial water reservoir Zalew Sulejowski located between Sulejów and Smardzewice. The Pilica floodplain is in many places sandy and dry, and its upper floodplain consists of sands and gravel. These make the terrain suited for forestry. The river valley has sandy alluvial soils, bog soils and patches of black soil.

Above the valley, there are lessivés, crypto-podzols and endoeutric cambisols (Baliński 1996).

A soil analysis was conducted in the study area (Mleczko, Ławrynowicz unpublished data). The material for the analysis was gathered in 2014 from 10 study plots in Spała and Konewka. The soils in the study area are acidic with mean pH in H_2O being 4,75 and 5,40 in Spała and Konewka respectively. The content of calcium in mg per 100 g of soil was significantly higher in Konewka than in Spała. The mean values for results of soil analysis are presented in Table 2 and the results are detailed in Appendix 2, Table 1.

Table 2. Mean values from soil analysis in the study area: pH in H_2O, pH in KCl, percentage of organic compounds (N, C and organic matter) and K_2O, P_2O_5, CaO, K, Na, Ca and Mg in mg per 100 g of soil.

study plots	pH in H_2O	pH in KCl	org. N	org. C	org. mat	K_2O	P_2O_5	CaO	K	Na	Ca	Mg
Spała	4,75	3,82	0,35	5,99	10,25	6,54	8,00	33,18	3,04	1,67	23,70	1,88
Konewka	5,40	14,34	0,21	3,62	6,28	13,87	15,24	73,92	9,34	2,48	52,80	3,20

3. MATERIALS AND METHODS

3.1. Subject of the study

As it was stated before, hypogeous fungi are a ecological, heterogeneous group which congregates various genera from Ascomycota (Elaphomycetales Pezizales), hypogeous 'gasteromycetes' from Basidiomycota (*Rhizopogon, Hymenogaster, Gautieria, Melanogaster*), and a few Glomeromycota (*Glomus* spp.). Some authors also include the genus *Scleroderma*, due to its morphological convergence with other hypogeous mushrooms (Castellano et al. 1989, Trappe et al. 2009). Though taxonomically distant from one another, groups of hypogeous fungi show features of convergent evolution in habitat adaptations, because they occupy a specific ecological niche, as mycorrhizal partners for plants, especially forest trees (Maser et al. 1978b; Ławrynowicz 1984; Maser, Maser 1988a; Luoma et al. 2003; Kirk et al. 2008; Hilszczańska et al. 2014). In fact, fungi with the hypogeous fruiting habit tend to dominate in mycorrhizal networks (Izzo et al. 2005). Most genera of hypogeous fungi can be directly related to epigeous genera, so the distinction between the two is not based on taxonomy. Hypogeous fungi include species which produce sporocarps underground, although some sporocarps can be found very close to the soil surface, partially submerged in the ground or in the leaf-litter (Ławrynowicz 1988; Pegler et al. 1993). Unlike epigeous mushrooms their spores cannot be released into the air and their main way of dispersion is by animals, particularly by insects and mammals. In case of hypogeous Ascomycota, the asci have no opening mechanisms and remain closed until natural decay or digestion by animals (Fogel, Peck 1975; Fogel, Trappe 1978; Maser et al. 1978a; Maser et al. 1985).

Hypogeous fruit bodies have mostly an unified structure. They form globose, subglobose or irregular sporocarps, with an inner spore-producing part called gleba, and a peridium consisting of an outer layer called cortex, and an inner peridium (Maser et al. 1978a ; Ławrynowicz 1988, Pegler et al. 1993). The peridium is the most nutrient-rich part of the sporocarp. As the sporocarp matures, the powdery mass of spores called gleba fills the whole fruit body. Ripe carpophores produce characteristic aromas, typical for given species and detectable by animals which feed on them (Trappe, Maser 1976; Maser et al. 1978a; Maser et al. 1978b; Taylor 1992; Johnson 1996; Maser et all 2008).

Hypogeous fungi tend to occur in large numbers in places called “oasis” or “nests”, where many different species form mycorrhizae with trees. Fungi fruit throughout the year, depending on the species and environmental conditions, and some species can even fruit in early spring under melting snow, but most species fruit only in a specific part of the year (Fogel 1976, 1981; Maser, Maser 1988a; Trappe et al. 2009). Seasonal abundance of hypogeous fungi follow changes in temperature and precipitation (Fogel 1976, Ure, Maser 1982; Luoma et al. 2003). Although annual abundance of sporocarps differ from season to season, as a group, they provide a stable food source for animals throughout the year (Maser, Maser 1988a).

The structure of the spores enables them to pass through the animal’s digestive tract with no alterations, and viable for further development outside the animal body (Trappe, Maser 1976; Maser et al. 1978b; Cork, Kenagy 1989; Claridge, Lindenmayer 1998; Claridge et al. 1999; Trappe, Claridge 2005; Trappe et al. 2009). Inside the animal, spores are exposed to body temperature, enzyme treatment and microorganisms, all of which might increase their ability to germinate and form mycorrhiza (Fogel, Trappe 1978). Additionally, animal pellets contain nutritional material and nitrogen-fixing bacteria, further enhancing fungal development (Li et al. 1986).

3.2. Studied animals

The bank vole *M. glareolus* (Schreber, 1780) (Wilson, Reeder 2005) is the most commonly spread rodent species in Poland. Its body length is 66-115 mm, tail: 35-63 mm and the weigh of the animal is 10,1-38,9 g (voles captured during this study ranged in weigh from 13,0 g to 30,5 g). Eyes are big, ears round, visibly protruding. The scull is massive, snout is round and short. Fur is red on the back, grey on the sides to white on the belly side (Pucek 1984).

The bank vole is a forest dwelling rodent, which occupies mixed and deciduous forests, parks, wooded river valleys and scrub-fields. It prefers dry and warm habitats. The individual territory ranges from 0,13 to 1,39 ha (Pucek 1984). The species is flexible in terms of habitat, adjusting to changing conditions (Ivanter 1975).

Bank voles climb well but live mostly close to the ground. They build round nests in old, rotten tree stamps (Pucek 1984), between roots and dig shallow, underground corridors which lead into the nest. The corridors are usually 5-10 cm deep and up to 10 m long. They go along tree roots, under a layer of moss, forest bed or snow

just next to the ground, or in existing crevices in a more rocky terrain (Ivanter 1975). A separate corridor branches out from the main corridor, leading to a hidden chamber where voles bring up their young. The chambers are usually 15-20 cm deep, but can reach even to 60 cm deep (Sokolov 1981). Whether the animals are active during the day depends on the temperature. In dry and warm periods, voles are active all day and night, in winter, the night activity is shortened, and in summer, the animals are less active during hot days, but more active during the night (Ivanter 1975).

The bank vole's diet is highly diverse and changes depending on food availability (Górecki, Gębczyńska 1962; Gębczyńska 1976; Hanssen 1985a; Maser, Maser 1988b). Voles feed mostly on seeds and fruits, but also on green parts of plants and on other animals: insects and other invertebrates (Pucek 1984). They generally do not eat grass, with their main food being forbs and forb-like green vegetation (Hanssen 1985a). Plant material contributes to 19-92% of the whole food intake, and animal food can contribute to 9-23% of the whole food intake, mostly in summer and winter (Sokolov 1981). Seeds are consumed often and in large quantities, and fungi are eaten when seeds are unavailable. Voles also have a high water consumption rate (Hanssen 1985a).

During spring, bank voles consume mostly green parts of vegetation (68% of the whole intake), seeds (Gębczyńska 1976), and insects (Holišová, Obrtel 1979). Similar patterns occur in summer, although the amount of seeds increases (from 5 to 11%). The amount of invertebrates consumed is also higher, with females consuming more animal food than males. In autumn green plant food makes up 38% of the whole intake, tree and herb seeds 40% and animal food 15%. The proportion holds through winter: green food - 38%, seeds - 56% and animal food - 6%. Bank voles also show a preference when eating green vegetation, depending on the developmental stage of a given plant (Gębczyńska 1976).

Bank voles are considered preferential mycophages (Rhodes 1986). They consume fungi, both with above- and under-ground fruit bodies (Drożdż 1966). During summer and autumn, fungi can contribute up to 20% - 30% of the whole food intake (Ivanter 1975), however, it is difficult to estimate the percentage of the overall production of fungi in the given ecosystem that is available to these rodents and is consumed by them (Drożdż 1966). Depending on the environment, fungi may dominate the diet. This is most common in case of coniferous forests (Hanssen 1985a). In deciduous forests, vole diet is dominated by forbs, tree leaves and tree seeds

(Holišová, Obrtel 1979). In managed forests, voles eat less seeds and fungi are consumed more frequently (Holišová 1971). Fungal material generally dominates when the abundance of grass, forbs, seeds, and insects is low (Drożdż 1966; Holišová 1971; Hansson, Larsson 1978; Hanssen 1985a; Ure, Maser 1982).

The yellow-necked mouse *A. flavicollis* (Melchior, 1834) is also associated with deciduous forests and found throughout Poland. It is bigger than the bank vole and of slightly different behaviour. It is 69-121 mm long, weighs 17-43 g (animals captured in this study weighted 12,0-46,5 g) and its tail measures 57-130 mm. The fur on the back is red-brown, belly distinctly white, with yellow spots on the breast area, sometimes creating a yellow collar. It inhabits old deciduous and mixed forests, blackberry and hazel thickets at clear-cuts. It prefers shaded and humid habitats (Pucek 1984).

The yellow-necked mouse is a good climber, active jumper and a good swimmer. It is mostly nocturnal and crediurnal (Nowak, Paradiso 1983). It builds its nests underground, under and between tree roots, in rocky crevices and in hollow trees.

The species feeds on seeds, green parts of plants and animal food, mainly arthropods. (Nowak, Paradiso 1983; Pucek 1984,). Unlike the bank vole, the yellow-necked mouse is less polyphagous, and eats mostly high-energy food (tree seeds and invertebrates) with less amount of fungal material in its diet (Górecki, Gębczyńska 1962; Drożdż 1966, 1968). Yellow-necked mice are more grainivores and seeds dominate in their diet throughout the year (Hansson 1985b). In spring yellow-necked mice consume high amounts of seeds, insects and green parts of plant. The quantities of seeds and insects then increase in summer, with plant material playing a marginal role in animal's diet (Górecki, Gębczyńska 1962). In autumn and winter, mice feed on tree seeds (Górecki, Gębczyńska 1962; Drożdż 1966, 1968).

The yellow-necked mouse is more aggressive and active than the bank vole. The mice are more often caught in live traps, as they penetrate them faster than the voles. Furthermore the vole will not return to a den which was taken over by the mouse, even when the mouse left (Sokolov 1981).

3.3. Sample gathering and preparation for analysis

The traps in both locations were set up approximately 5-10 meters apart, mostly in shaded and well covered spots. Every spot was assigned a number, 1-30 for

Spała and 1-20 for Konewka. Trappings were conducted in a sequence of a 4 to 5 days and nights in May (26-30 V 2014, 18-21 V 2015), July (5-11 VII 2013, 21-25 VII 2014) and October (14-18 X 2013, 13-16 X 2014). The traps were checked 3 times a day approximately every 6 hours: at sun rise, midday and late evening. The traps were left open through the night. A mixture of seeds, oat, sunflower and sesame was used as bait.

Rodents were determined to the species level. After the capture, animals were weighed and their sex and age group (juvenile or adult) were determined for later comparisons. Also, the trap's number was noted. The animals were marked with a red dot on the abdomen. For a dye, a water solution of red henna was used, due to its bright colour, long lasting and no toxic effects for the animals. After marking, the animal was released. A faecal sample was then taken from the live trap and placed in a 1,5 ml Eppendorf tube with 1 ml of 90% ethanol for preservation. The samples were labelled with a number of the animal caught in this particular trapping.

In the laboratory, faecal samples were prepared for microscopic analysis. The preparation method was based on methods presented by Claridge, Lindermayer (1998), Colgan, Claridge (2002), Bertolino et al. (2004) and Kataržytė, Kutorga (2011).

Each sample consisted of overall material gathered from one animal captured in a live trap. Samples were cleared of food remnants, dried and weighted with RADWAG WPE 30S weight (temperature range 50-140 ^{o}C, dt=2 ^{o}C, humidity 0,2-100%, dw=0,1%). Next, each sample was crushed in a mortar and put in a 1,5 ml Eppendorf tube with 1 ml 90% ethanol. The tubes were then centrifuged using the Hettich Zentrufugen EBA 21 at speed of 15000 rounds per minute for 2,5 minutes. Approximately 0,1 ml of slurry was placed on the microscopic glass, along with a drop of Meltzer reagent, covered and placed under a microscope. Samples were examined using NIKON E200 light microscope under x600 magnification. Spores of hypogeous fungi which were found in samples were determined to genus level using identification keys: Błaszkowski (2012), Castellano et al. (1989), Hawker (1954), Ławrynowicz (1979, 1988), Pegler et al. (1993). Determination to a species level was preformed if the morphological characteristics enabled a certain identification.

Spores observed under the microscope were photographed for documentation, using NIKON D90 Digital Camera.

Spores were identified based on four traits: shape, size, colour and ornamentation. Size was measured by width and length of the spore.

After successful spore identification, a drop of slurry from each positive sample was put in Bürker slide with a drop of Meltzer reagent (aqueous solution of chloral hydrate, potassium iodide and iodine). For each sample, spores were counted in ten 0,04 mm^2 grids of the Bürker slide. The number of spores used in the statistical analysis was estimated as the sum of spores counted in ten grids of the Bürker slide for each sample. At first the number of spores was calculated for the a ml of suspension in the Bürker slide using the appropriate formula, but the author later switched back to raw spore number (sum of spores in ten grids). This was due to calculation convenience, as the results of statistical testing on number of spores and number of spores per ml differed only in order of magnitude and not in statistical significance.

3.4. Statistical methods

Differences in temperature between seasons was tested with ANOVA I. Generalized linear model (GLM) with Poisson distribution and identity link function was used to describe the relationship between hypogeous fungi spores' number, rodent species, sexes, age group (juvenile, adult), study plot (Spała and Konewka) and weather conditions. Weather conditions during and prior to the trappings were taken into account. In order to reduce the number of dimensions factor analysis with varimax normalized was performed, and two first components (PCA_1 - mainly attributed to temperature and humidity and PCA_2 - mainly attributed to rainfall and wind, see Appendix Table 1) were taken for further analysis. The model was built using the general linear methods: best subsets method, general custom designs, quick specs dialog and sigma-restricted parametrization. The sum of squares was counted using the regression method. The best fitting model was chosen using the Akaike information criterion (AIC). The interactions between rodent species, sexes and age were analysed using a cross model with a between effect.

The GLM with Poisson distribution was also used to analyse if there was a connection between mean spore number and the trap's distance from the road. The distance was presented in the following logarithmic-normal scale:

1 – distance up to 1 m from the road;

2 – distance up to 2 m from the road;

3 – distance from 3 to 7 m from the road;

4 – distance from 8 to 20 m from the road;

The border values of the scale were calculated using the formula – e^k (with Euler's constant *e*, and *k* being the next upper interval of the scale. The first category was separated to two (1-2) for better results.

Assuming that in both animal populations there are two feeding strategies – foraging and not foraging on fungi – it can be presumed that individuals which search actively for sporocarps will not only have bigger diversity of spores in faecal samples but also will have more spores in them. Due to this samples with spores present in them were qualified into one of three classes:

I class – low number of hypogeous and epigeous spores (1-30% of examined non-overlapping view areas)

II class – medium number of hypogeous and epigeous spores (35-65% of examined non-overlapping view areas);

III class – high number of hypogeous and epigeous spores (>70% of examined non-overlapping view areas).

The numbers of hypogeous and epigeous spores were qualified separately. For convenience the classes are hereafter referred to as "first - third hypogeous class" and "first - third epigeous class". Samples with no spores in them are referred to as "zero hypogeous class" and "zero epigeous class".

To see if there are differences in the spore number class among the animals species in study areas, a Pearson χ^2 test was performed. Also, the number samples in each class of hypogeous and epigeous spores were compared and tested for correlation.

Assuming that the presence of fungal spores in gathered samples is the expression of the abundance of sporocarps in animal diet, the information on the number of species and individual spores were used to count Shanon's diversity index for each sample. The index was calculated according to the formula:

$$H' = -\sum_{i=1}^{S} (p_i \times \ln p_i)$$

H' – Shanon's diversity index,

p_i – proportion of the given taxon in the given sample, in relation to the number of all registered spores in the given sample,

S – number of all identified taxa in all samples.

In this analysis a variant of the index based on the natural logarithm was used (with entropy expressed in nats, not bits). In theory Shanon's diversity index reaches values from 0 to ∞, but in practice of ecological studies it usually has values for 0 to 3-

4. The growing value of Shanon's index corresponds to the growing diversity of the environment.

Based on Shanon's diversity index, an effective taxa number was calculated, according to the formula:

$$E = e^{H'}$$

E – the effective number of species,

e – Euler's constant,

H' – Shanon's diversity index.

This value enables the practical interpretation of the calculated diversity, in accordance with the principle that a community (in this case a sample) of an effective number of taxa E, has an equal diversity to a community with real number E of equinumerous taxa. This description allows to extrapolate the evaluation of diversity to the samples of a zero number of identified taxa, through an assumption that in those samples, the effective number of taxa $E = 0$.

The distribution of index values were tested for normality: Shapiro-Wilk test, Shapiro-Franca test, D'Agostino tests based on skewness, kurtosis and both moments (D'Agostino-Pearson test), Lillefors test, Cramer-von Mises test, Anderson-Darling test, Chi-squared test and a modification of data driven Neyman normality test. Distributions of index values for *M. glareolus* and *A. flavicollis* were analysed for monotonicity of kernel density estimation function, and for this purpose the first numerical derivative for both distributions was calculated.

Preferences in animal diet were estimated using the indices for dominance, frequency and ecological importance (Kasprzak, Niedbała after Czachorowski 2006), according to three formulas:

$$P_i = \frac{\sum_{j=1}^{N} p_{i,j}}{N}$$

P_i – dominance index for *ith* taxon

$p_{i,j}$ – proportion of spores of i taxon in the *jth* sample

N – number of samples in the class for which the dominance index is calculated (I-III)

$$F_i = \frac{N_i}{N}$$

F_i – frequency index for *ith* taxon

N_i – number of samples in which the given taxon was identified

N – number of samples in the class for which the dominance index is calculated (I-III)

$$Q_i = \sqrt{P_i \times F_i}$$

Q_i – contribution index for *ith* taxon

P_i – dominance index for *ith* taxon

F_i – frequency index for *ith* taxon

The size of spores observed under the microscope was noted for calculating the circularity index (length/width), descriptive statistics and ANOVA I testing for changes in spore size between seasons.

Statistical analysis was done using Statistica 10.0 package and the R statistic package. Values of 95,00 % confidence interval (CI) and statistical significance p=0,05 were applied in the analysis.

4. RESULTS

4.1. Gathered samples

Overall 247 samples were examined: 196 from yellow-necked mouse, and 51 from bank vole. 166 samples were gathered from Spała (131 from yellow-necked mouse and 35 from bank vole), and 81 from Konewka (65 from yellow-necked mouse and 16 from bank vole). Detailed numbers of samples gathered are presented in Table 3.

Table. 3. Overall samples gathered from *Apodemus flavicollis* and *Myodes glareolus* from Spała and Konewka reserves in years 2013-2015 in May, July and October.

	Spała			Konewka		
Animal	MAY	JULY	OCTOBER	MAY	JULY	OCTOBER
A. falvicollis	32	47	52	15	26	24
M. glareolus	5	23	7	3	10	3

From the overall number of samples, spores of hypogeous fungi were found in 94 samples (65 from yellow-necked mouse and 29 from bank vole) and in 35 samples more than one genus was found (in 22 samples from yellow-necked mouse and 13 samples of bank vole). Spores of epigeous fungi were found in 235 samples and only 9 samples no spores were detected. Details on the positive samples and spores found in them are shown in Tables 4 and 5.

Table. 4.Fungal genera found in samples gathered from *Apodemus flavicollis* and *Myodes glareolus* from Spała and Konewka reserves in years 2013-2015 in May, July and October. The presented number is the number of positive samples where the given genus was found.

	MAY				JULY				OCTOBER			
	A. flavicollis		*M. glareolus*		*A. flavicollis*		*M. glareolus*		*A. flavicollis*		*M. glareolus*	
	Spała	Konewka	Spała	Konewka	Spała	Konewka	Spała	Konewka	Spała	Konewka	Spała	Konewka
Elaphomyces	4	1	0	1	0	0	1	0	0	0	0	0
Hydnotrya	2	1	0	0	1	3	1	1	2	0	0	1
Pachyphloeus	0	0	0	0	0	0	0	0	0	0	0	1
Genea	0	0	0	0	2	0	0	0	0	0	0	0
Tuber	0	0	0	0	1	0	1	0	0	0	1	0
Hymenogaster	3	2	0	0	4	6	0	1	1	1	0	0
Melanogaster	0	0	1	0	5	2	0	0	1	0	0	0
Rhizopogon	2	0	0	0	4	1	8	2	1	0	0	0
Scleroderma	0	0	0	0	3	2	0	0	0	1	0	0
Gautieria	0	0	0	0	2	0	0	0	1	0	0	0
Glomus	0	0	1	0	3	0	6	1	0	1	1	0
Endogone	0	0	0	0	0	1	0	0	1	0	0	0

Table. 5.The overall number of spores found in positive samples gathered from *Apodemus flavicollis* and *Myodes glareolus* from Spała and Konewka reserves in years 2013-2015 in May, July and October, with N (the sum of spores in 10 squares of Bürker chamber per sample), and the percentage of the sum of all spores (625 - 100%)

	MAY				JULY				OCTOBER			
	A. flavicollis		*M. glareolus*		*A. flavicollis*		*M. glareolus*		*A. flavicollis*		*M. glareolus*	
	Spała (N; %)	Konewka (N; P)	Spała (N; P)	Konewka (N; P)	Spała (N; P)	Konewka (N; P)	Spała (N; P)	Konewka (N; P)	Spała (N; P)	Konewka (N; P)	Spała (N; P)	Konewka (N; P)
Elaphomyces	23; 3,7	14; 2,2	0; 0,0	1; 0,2	0; 0,0	0; 0,0	11; 1,8	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0
Hydnotrya	4; 0,6	1; 0,2	0; 0,0	0; 0,0	1; 0,2	12; 1,9	3; 05	6; 1,0	2; 0,3	0; 0,0	0; 0,0	1; 0,2
Pachyphloeus	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	1; 0,2
Genea	0; 0,0	0; 0,0	0; 0,0	0; 0,0	15; 2,4	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0
Tuber	0; 0,0	0; 0,0	0; 0,0	0; 0,0	1; 0,2	0; 0,0	1; 0,2	0; 0,0	0; 0,0	0; 0,0	1; 0,2	0; 0,0
Hymenogaster	22; 3,5	14; 2,2	0; 0,0	0; 0,0	103; 16,0	42; 6,7	0; 0,0	4; 0,6	1; 0,2	8; 1,3	0; 0,0	0; 0,0
Melanogaster	0; 0,0	0; 0,0	1; 0,2	0; 0,0	41; 6,6	2; 0,3	0; 0,0	0; 0,0	2; 0,3	0; 0,0	0; 0,0	0; 0,0
Rhizopogon	4; 0,6	0; 0,0	0; 0,0	0; 0,0	35; 5,6	1; 0,2	168; 27,0	16; 2,6	2; 0,3	0; 0,0	0; 0,0	0; 0,0
Scleroderma	0; 0,0	0; 0,0	0; 0,0	0; 0,0	3; 0,5	2; 0,3	0; 0,0	0; 0,0	0; 0,0	1; 0,2	0; 0,0	0; 0,0
Gautieria	0; 0,0	0; 0,0	0; 0,0	0; 0,0	8; 1,3	0; 0,0	0; 0,0	0; 0,0	1; 0,2	0; 0,0	0; 0,0	0; 0,0
Glomus	0; 0,0	0; 0,0	9; 1,4	0; 0,0	6; 1,0	0; 0,0	26; 4,2	1; 0,2	0; 0,0	1; 0,2	1; 0,2	0; 0,0
Endogone	0; 0,0	0; 0,0	0; 0,0	0; 0,0	0; 0,0	1; 0,2	0; 0,0	0; 0,0	1; 0,2	0; 0,0	0; 0,0	0; 0,0

4.2. Seasonal changes in spore numbers

The mean number of spores and the average temperature was used for testing significant differences between the seasons. The testing has shown that the mean spore number was significantly correlated with the mean temperature of the month (r=0,999; df=1; p<0,05, 95,00 % CI). In July, when the mean air temperature was the highest (19,22°C), the mean number of spores was also the highest (8,80) (Fig. 1).

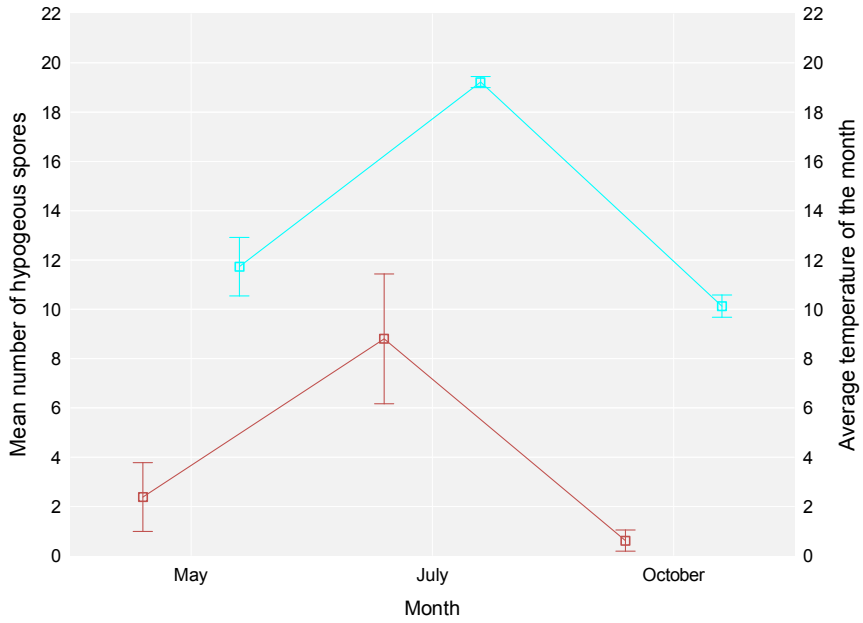


Fig. 1. Box plot showing the mean number of spores (the red line, df = 2; Wald stat. = 767,741; p < 0,0001) and the average temperature of the month (dotted blue line, $F_{(2;228)}$ = 474,9781; p < 0,0001).

The factor analysis with the AIC has shown that the best model describing seasonal changes in spore number in samples, is the model taking into account the component 1 (PC_1 - temperature and humidity), component 2 (PC_2 - rainfall and wind speed), the study plot, and the rodent species (see Appendix Table. 2). The mean number of spores was positively related to PC_1 and negatively related to PC_2 associated with "stormy conditions". Higher values for rainfall, wind and humidity were related to lower spore number in samples (Table 6, see also Appendix Table 3). The analysis with weather conditions from months prior to trappings did not yield statistically significant results.

Table. 6.Analysis of diversity of fungal spores in relation to weather conditions (PCA_1 ,PCA_2), study plot and rodent species (statistically significant values marked with red colour).

effect	df	Wald Stat.	p
intercept	1	920,68	<0,0001
PCA_1	1	834,20	<0,0001
PCA_2	1	101,78	<0,0001
study plot	1	707,69	<0,0001
rodent species	1	241,81	<0,0001

4.3. Differences in spore numbers between study plots

Generally, the mean number of examined spores was higher in Spała (6,70) than in Konewka (3,70) (Fig. 2). The mean number of ascomycetes spores was significantly higher in Spała than in Konewka. The mean numbers of spores of basidio- and glomeromycetes was not so strongly connected with the study area as with the weather conditions.

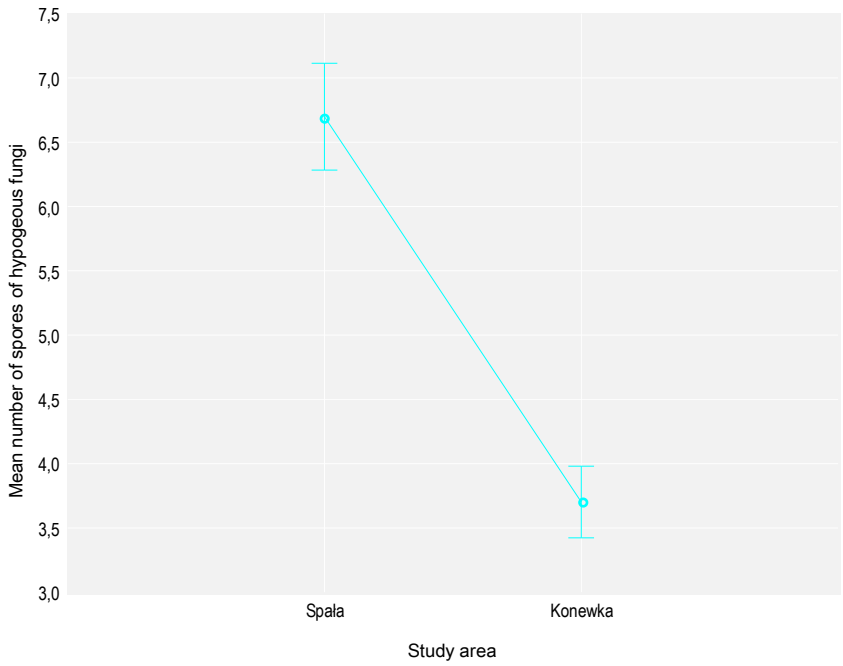


Fig. 2. Box plot showing the variation of mean number of spores of hypogeous fungi in both study areas, with statistical significance (df=1; Wald stat. = 707,69, p<0,0001).

4.4. Differences between species, sexes and age groups

Mean numbers of spores of asco-, basidio- and glomeromycetes were related with the animal species, but not so strongly as with weather conditions and the study area (see Appendix Table 4-6). Because the three spore groups (asco, basidio- and glomeromycetes) maintain a similar relations within the factor analysis, the groups were merged together, for a better statistical sample.

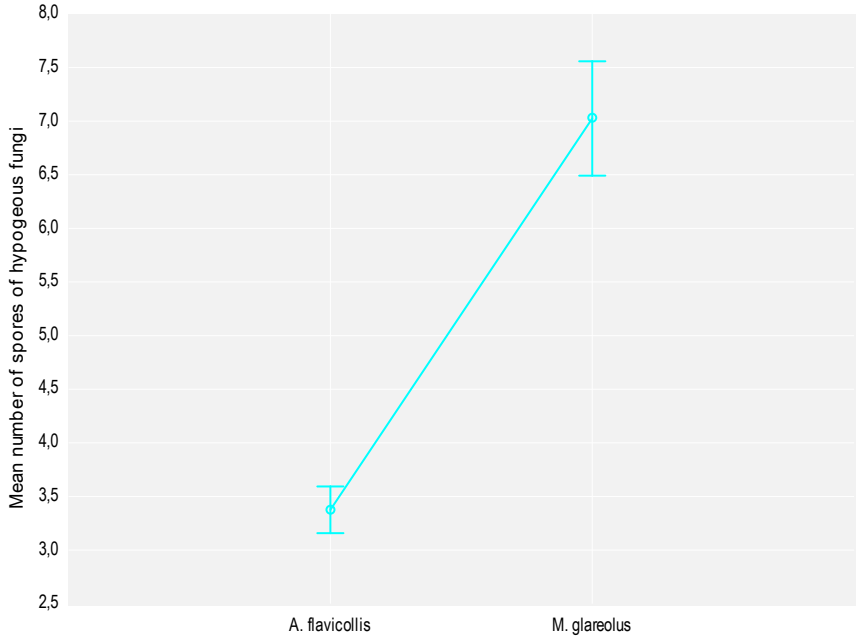


Fig. 3. Box plot showing the variation of mean number of spores of hypogeous fungi between *A. flavicollis,* and *M. glareolus*, with statistical significance (df=1; Wald stat. = 241,81, p<0,0001).

There was a significant difference between the animal species in the mean number of spores found in faecal samples. The mean number was significantly higher in the bank vole samples than in samples from yellow-necked mouse (Fig. 3). Among the yellow-necked mice there was a significant difference in mean spore number between sexes and between age groups of animals, with females and juveniles having significantly higher mean number of spores in their faecal samples (Table 7-8, Fig. 4-5). Such differences were not noted among the bank voles.

Table 7. Analysis of diversity of fungal spores in relation to rodent species and sex (statistically significant values marked with red colour).

effect	df	Wald Stat.	p
intercept	1	594,46	0,000
species	1	50,79	0,000
sex	1	5,25	0,022
species × sex	1	5,55	0,019

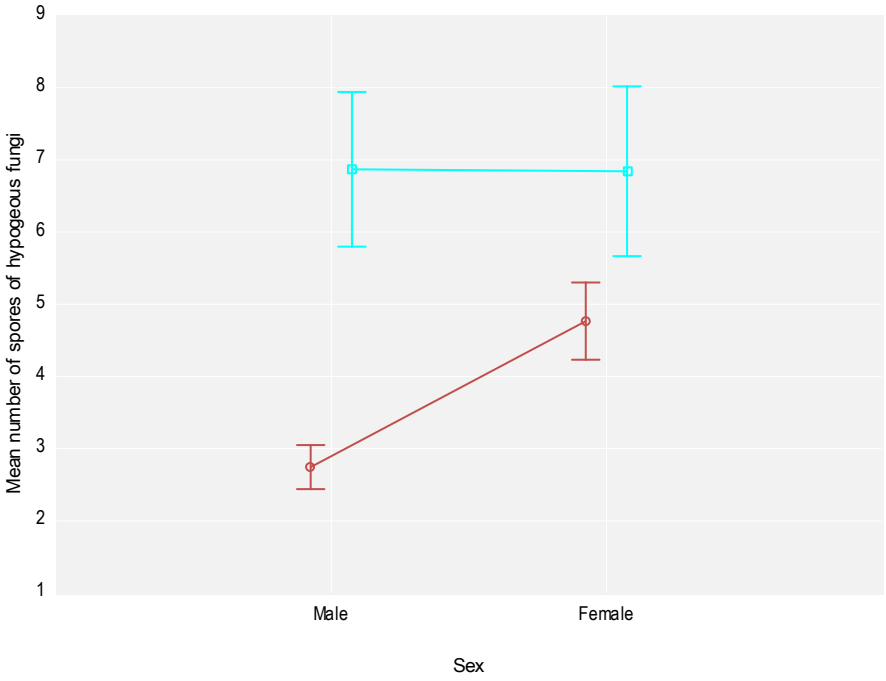


Fig. 4. Box plot showing the variation of mean number of spores of hypogeous fungi between sexes of *A. flavicollis* (red box plot), and *M. glareolus* (blue box plot), with statistical significance.

Table 8. Analysis of diversity of fungal spores in relation to rodent species and age (statistically significant values marked with red colour).

effect	df	Wald Stat.	p
intercept	1	707,33	0,000
species	1	114,67	0,000
age	1	90,58	0,000
species × age	1	5,95	0,015

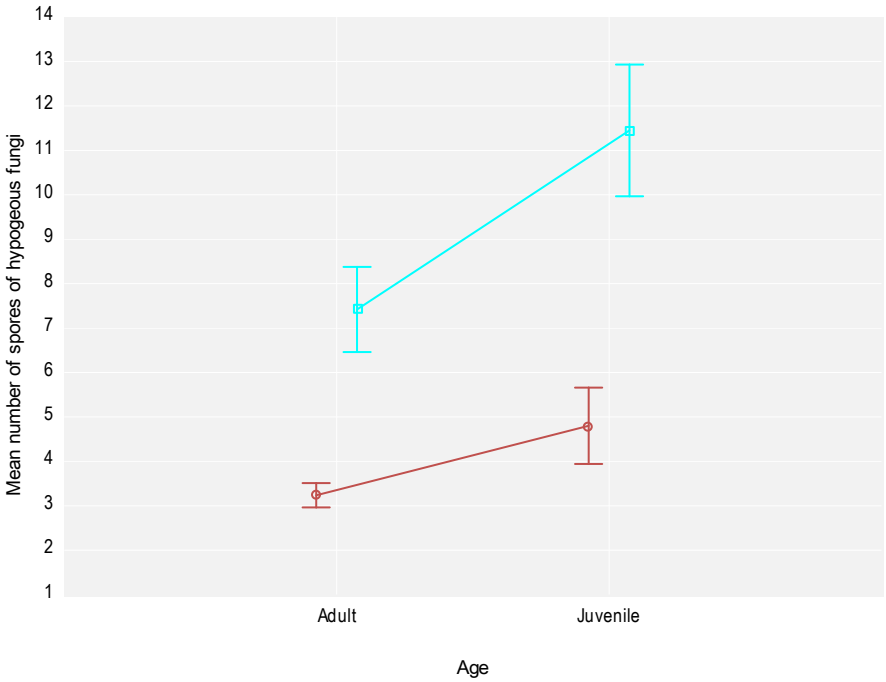


Fig. 5. Box plot showing the variation of mean number of spores of hypogeous fungi between adults and juveniles of *A. flavicollis* (red box plot), and *M. glareolus* (blue box plot), with statistical significance (df=1; Wald stat. = 90,58, p=0,000).

4.5. Spore numbers in relation to trap's distance from the road

There was a significant relation between the mean number of spores and the distance from the given trap to the road. More spores were found in samples which were taken from traps set 1-3 m from the road, than in those taken from traps located further in the forest (Table 9, Fig. 6).

Table 9. Analysis of diversity of fungal spores in relation to study plot and distance from the road (statistically significant values marked with red colour).

effect	df	Wald Stat.	p
intercept	1	675,15	0,000
study plot	1	68,67	0,000
distance from the road	2	100,28	0,000

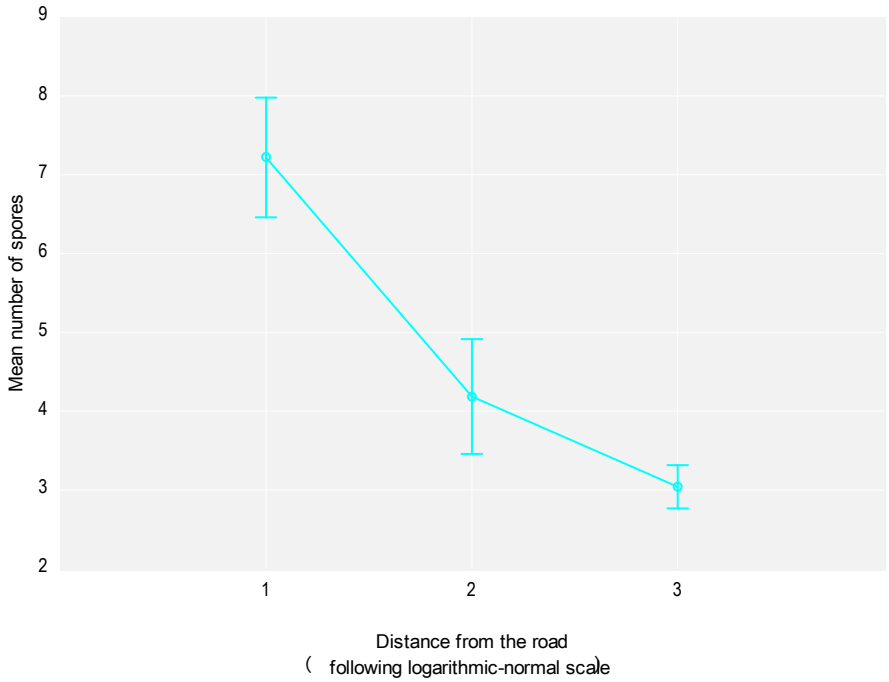


Fig. 6. Box plot showing the mean number of spores of hypogeous fungi in relation to the trap distance from the road (fallowing logarithmic-nominal scale, see chapter 3.4 Statistical methods) (Wald stat.=100,28, p=0,000, 95,00% CI)

4.6. Hypogeous vs. epigeous fungi

Comparing number classes for hypogeous and epigeous fungi shows that the majority of overall samples (159) did not have hypogeous spores in them, but among those only 9 did not have epigeous spores. 92 samples of the zero hypogeous class had the first epigeous class, and 41 and 17 had second class and third class, respectively. In samples with the third hypogeous class 0, 3, 10 and 6 samples were in the zero, first, second and third epigeous classes, respectively (Table 10).

Table 10. Overall number of samples with each number class (0-3).

hypogeous	0 epigeous	1st epigeous	2nd epigeous	3rd epigeous	total
0	9	92	41	17	159
1	3	24	14	12	53
2	0	5	8	3	16
3	0	3	10	6	19
total	12	124	73	38	247

Analysis of the number classes found in both animal species showed that most faecal samples had either zero or first hypogeous class. The number of zero class was evident in *A. flavicollis* (90 of 131 analysed samples from Spała, and 47 out of 65 from Konewka) and was a little more balanced in *M. glareolus* (14 out of 35 from Spała and 8 out of 16 from Konewka). In order to see if there was a correlation between the classes, the Gamma test was used. Due to small numbers of samples, the correlation test could be undertaken only for the overall number, without dividing samples according to study area, animal species or season. For N=247 and p<,05000, the result of the Gamma test (Gamma=0,384658, Z=5,523144), showed that indeed, there was a positive correlation between hypo- and epigeous classes. Because of the high amount of zero class samples, the correlation could not be tested with the Pearson χ^2 test for significance. After reducing the number of hypogeous classes to two, indicating presence or absence of spores, the Pearson χ^2 tests showed that there was a statistical significance for the correlation (Pearson χ^2=14,339, df=3, P=0,002).

4.5. Analysis of taxa diversity in samples

Histograms of empiric distributions of Shanon's diversity index for hypogeous taxa in samples from *A. flavicollis* and *M. glareolus* (Fig. 7), show that indices are not of normal distribution. This is confirmed by results of ten normality tests shown in Table 11.

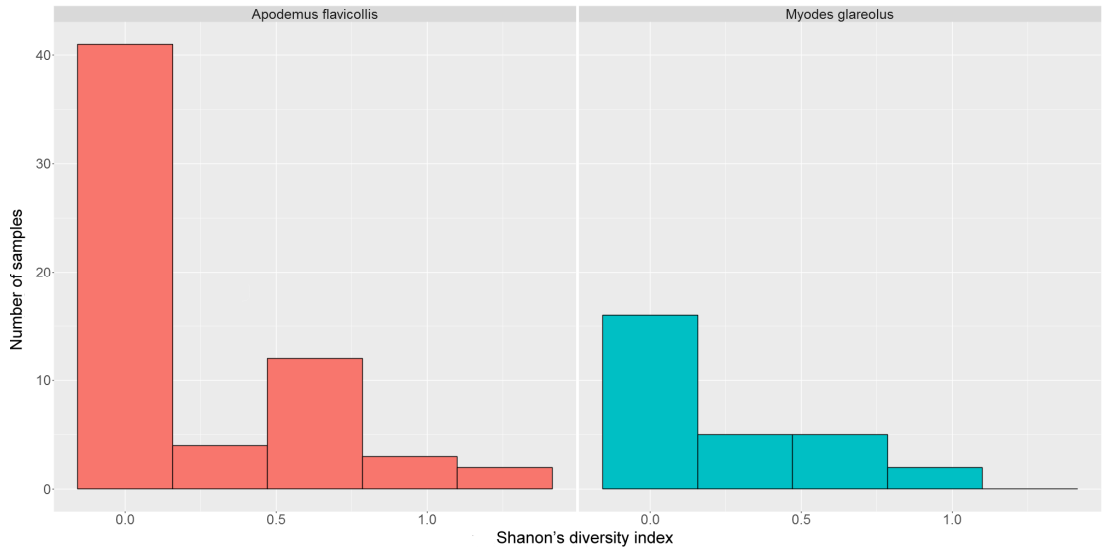


Fig. 7. Histograms of empiric distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis* (red) and *M. glareolus* (blue).

Table 11. Critical probabilities for results of ten normality tests for empiric distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis*, and *M. glareolus*.

normality test	*Apodemus flavicollis*	*Myodes glareolus*
Shapiro-Wilk test	$2{,}834 \times 10^{-10}$	$1{,}486 \times 10^{-5}$
Shapiro-Francia test	$7{,}068 \times 10^{-9}$	$7{,}260 \times 10^{-5}$
X^2 Pearson test	$9{,}499 \times 10^{-51}$	$4{,}725 \times 10^{-11}$
Liliefors test	$2{,}522 \times 10^{-28}$	$3{,}480 \times 10^{-9}$
date driven Neyman normality test	$4{,}121 \times 10^{-9}$	$6{,}015 \times 10^{-4}$
D'Agostino test based on skewness	$2{,}200 \times 10^{-4}$	$3{,}066 \times 10^{-2}$
D'Agostino test based on kurtosis	$3{,}280 \times 10^{-1}$	$5{,}767 \times 10^{-1}$
D'Agostino-Pearson test	$6{,}731 \times 10^{-4}$	$8{,}278 \times 10^{-2}$
Cramer-von Mises test	$7{,}370 \times 10^{-10}$	$9{,}058 \times 10^{-7}$
Anderson-Darling test	$4{,}201 \times 10^{-22}$	$7{,}021 \times 10^{-8}$

Figure 8 shows critical statistical significance of the tests. With the standard probability p=0,05, only the tests of D'Agostino based on kurtosis and D'Agostino-Pearson did not allow to reject the H_0 hypothesis for the normality of distribution.

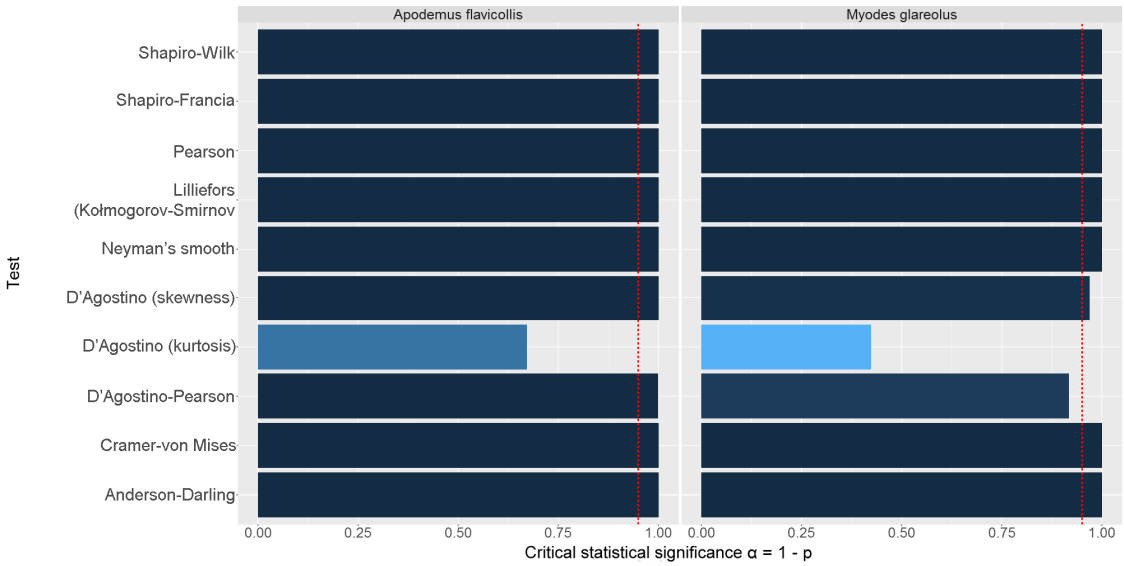


Fig. 8. Critical statistical significance of 10 normality tests for distributions of Shanon's diversity index for fungal taxa in faecal samples from *A. flavicollis* and *M. glareolus* (the red dotted line indicates the significance α = 0,95 corresponding to the standard probability p = 0,05).

In this analysis samples with no spores identified were not taken into account, because of the inability to calculate Shanon's index. The empiric distributions of Shanon's index for the samples with at least one fungal taxon are clearly asymmetrical to the right side, but approximately normokurtic. The measures of asymmetry and concentration of the distributions are presented in Table 12. The observed distributions

are also multimodal – at least bimodal. First dominants D_0 of both distributions are equivalent to general dominants equal 0, and correspond to samples with only one identified taxon.

Table 12. Values of asymmetry and concentration measures for distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis* and *M. glareolus* (the fourth column contains values for an ideally normal distribution)

measure	*Apodemus flavicollis*	*Myodes glareolus*	normal distribution
third central moment (μ_3)	0,0615	0,0207	0,0
Pearson's asymmetry factor μ_3/σ^3	1,2685	0,9279	0,0
Standard error kurtosis μ_4/σ^4	3,3728	2,3564	3,0

By analyzing both distributions for monotonicity of kernel density estimation function and calculating the first numerical derivative, it was possible to estimate the second dominant in both distributions (Figs 9-10). The new dominants D_1 are equivalent to the third zero moments of the derivatives and amount at 0,591 nat for distribution of samples from *A. flavicollis*, and 0,540 nat for distribution of samples from *M. glareolus*. These values correspond to samples with the effective taxa number E_{Af} = 1,805 and E_{Mg} = 1,716 respectively. This suggests that in both cases the distributions consist of two sub-distributions – one with D_0 = 0 is similar to Poisson's distribution, and the other one with the new dominant D_1 is more like the normal distribution. It was impossible to separate fully the sub-distributions, as the statistical methods (eg. Bhattachary's method, Gregor's method) enable only separation of bimodal distributions consisting of two normal distributions and not a mix of Poisson's and normal distribution.

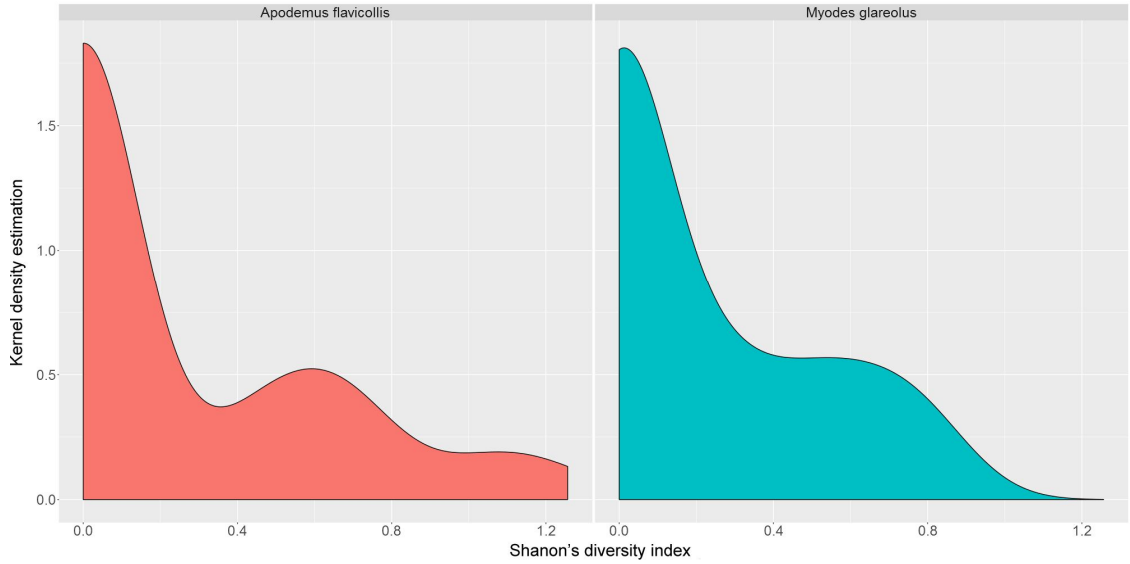


Fig. 9. Graphs of the kernel density estimations for empiric distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis* (red) and *M. glareolus* (blue) (the kernel is the function of density of Gauss probability, the smoothness parameter selected by the Silverman method).

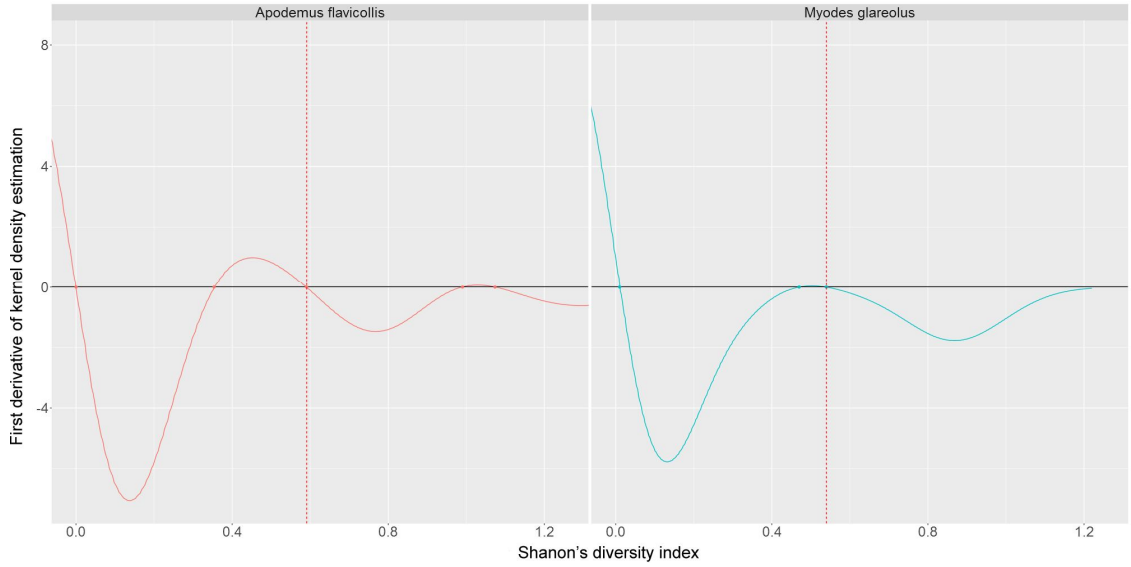


Fig. 10. First numerical derivatives of kernel density estimatior for empiric distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis* (red) and *M. glareolus* (blue) (the red dotted line indicates the zero moment of the derivative, which corresponds to the second dominants of the distributions).

Figure 11 shows the results of 8 normality tests for Shanon's distribution in the number classes. Two of the previously used tests – the D'Agostino test based on kurtosis and the D'Agostino-Pearson test – could not be used due to insufficient number of samples. It is clear that in classes with higher number of spores the distributions of Shanon's index are more like the normal distribution. In the III class, all conducted tests did not allow to reject the H_0 hypothesis for normality of distribution.

Thought it was impossible to separate completely the sub-distributions and their corresponding sub-populations in both rodent species, some tendencies in feeding on hypogeous fungi were found in classes of rodents that have higher values of the number of spores in their faecal samples.

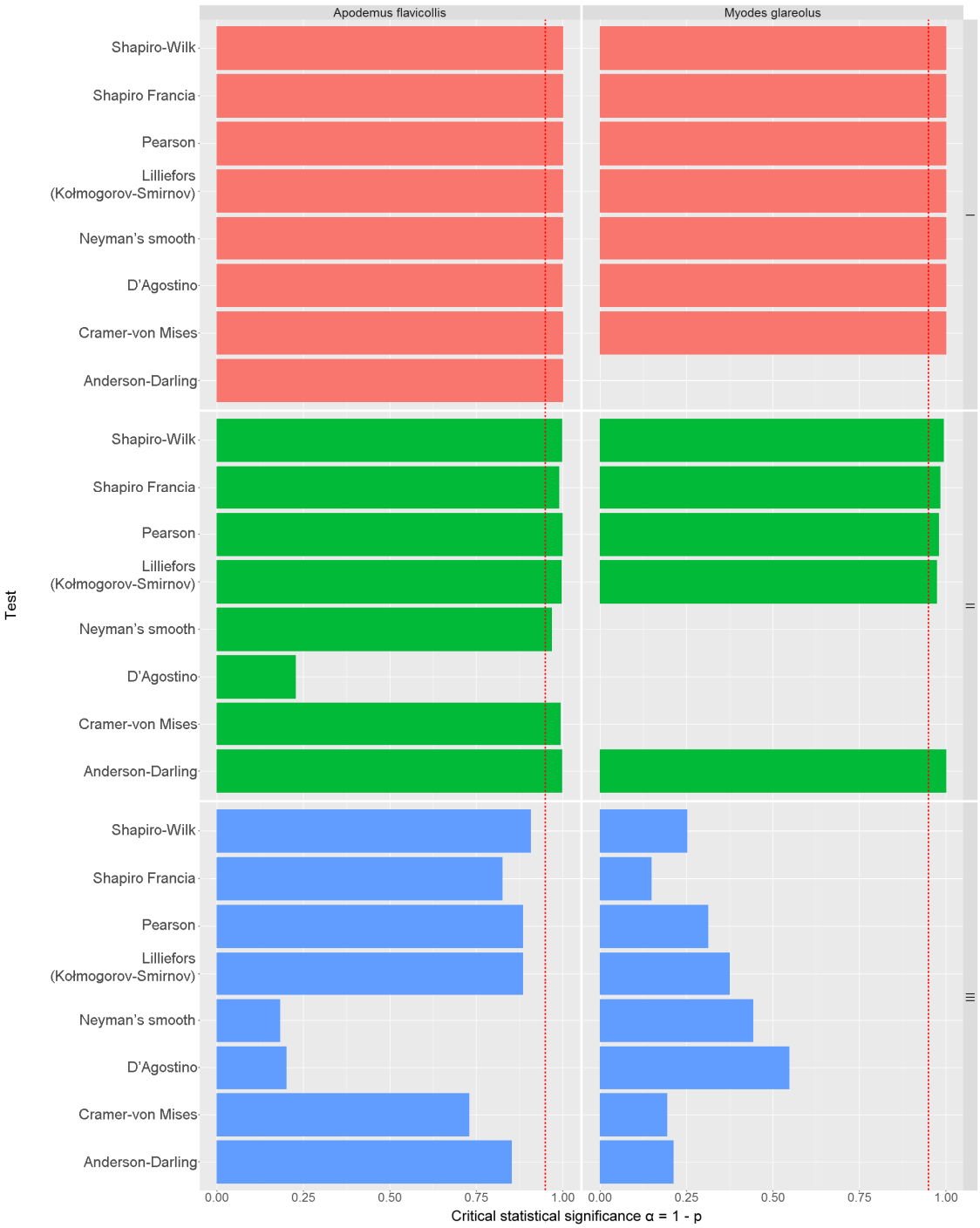


Fig. 11. Critical statistical significance of 8 normality tests for distributions of Shanon's diversity index for hypogeous taxa in faecal samples from *A. flavicollis* and *M. glareolus* divided into hypogeous classes defined by the number of spores found in samples (the red dotted line indicates the significance $\alpha = 0{,}95$, corresponding to the standard probability $p = 0{,}05$). Some tests could not be conducted due to insufficient number of samples (compare Fig. 8).

4.6. Fungal genera in relation to number classes

Figures 12-14 respectively show the indices for dominance, frequency and ecological importance for spores of hypogeous fungi divided into number classes. For a better comparison the studied taxa of fungi are sequenced in accordance to the descending values of the dominance index (Fig. 12) in samples with the first hypogeous class from *A. flavicollis*.

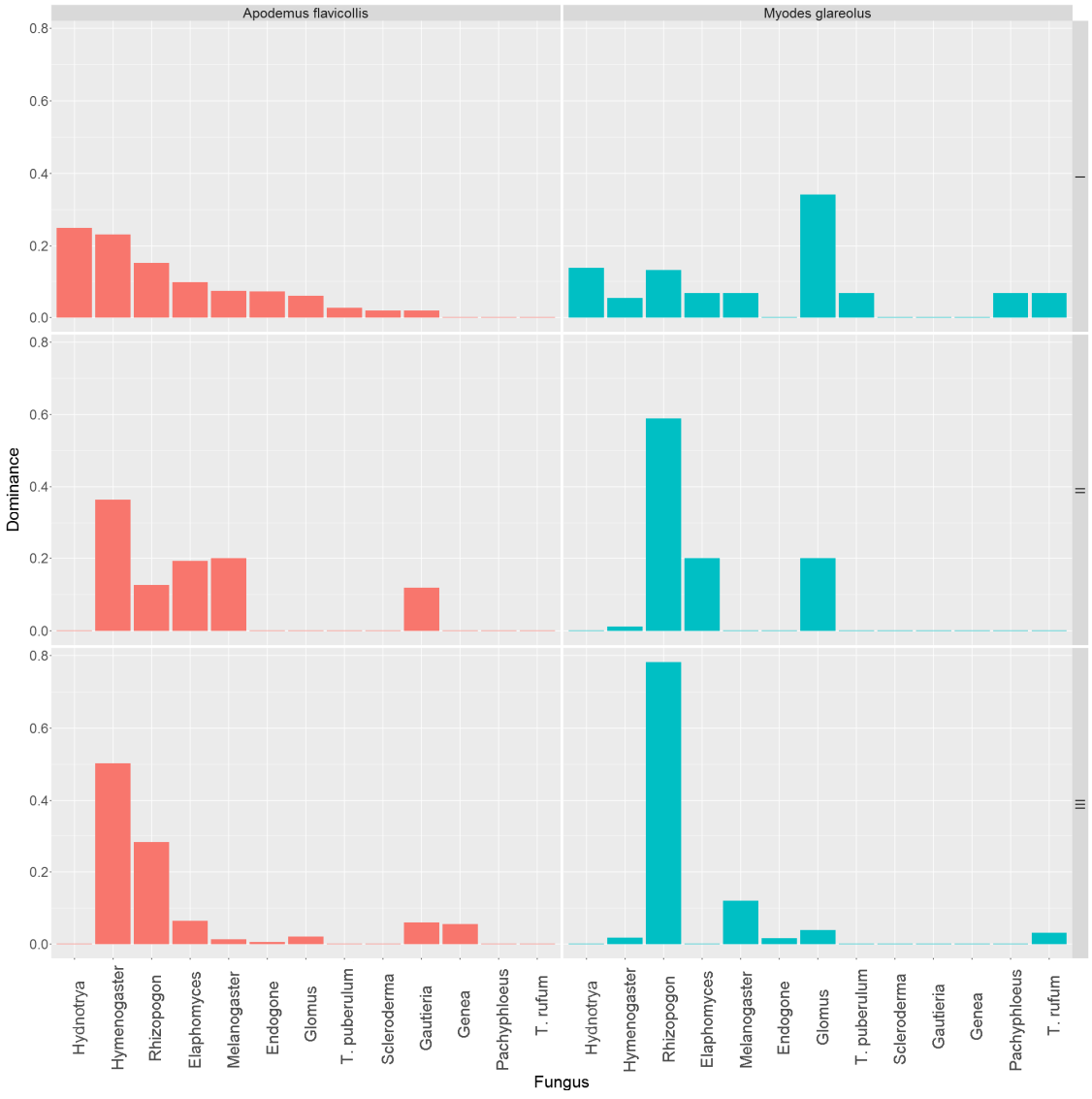


Fig. 12. Dominance indices of hypogeous fungi taxa in samples from *A. flavicollis* and *M. glareolus,* divided into number classes. The sequence of the fungal taxa corresponds to the descending values of the dominance index in samples of the I class from *A. flavicollis*.

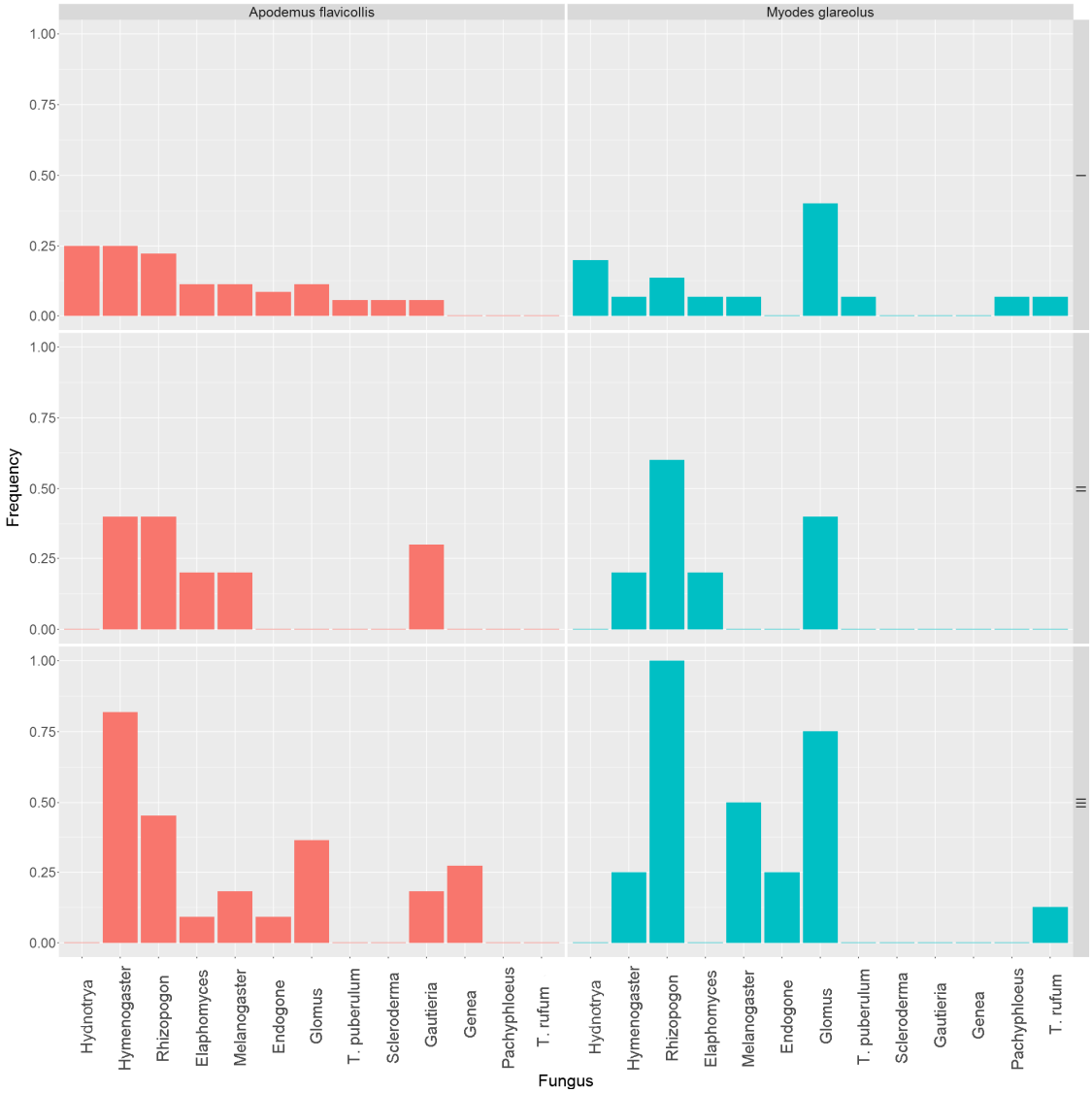


Fig. 13. Frequency indices of hypogeous fungi taxa in samples from *A. flavicollis* and *M. glareolus,* divided into number classes. The sequence of the fungal taxa corresponds to sequence used in Fig. 12.

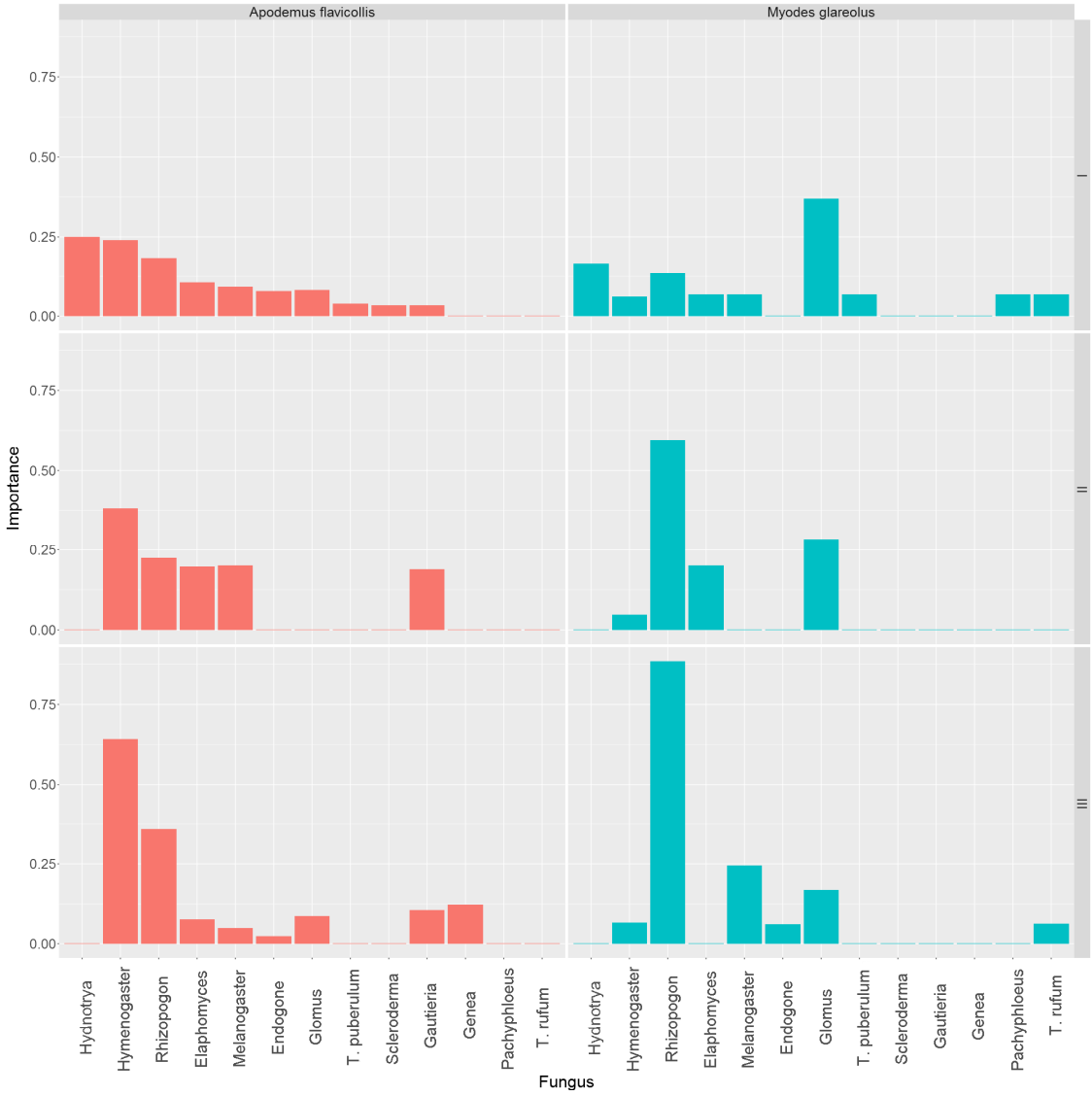


Fig. 14. Ecological importance indices of hypogeous fungi taxa in samples from *A. flavicollis* and *M. glareolus,* divided into number classes. The sequence of the fungal taxa corresponds to sequence used in Fig. 12.

The index of dominance, frequency and ecological importance among *A. flavicollis* of the first class show that there is low diversity in the spore taxa, with the values ranging from 0,0 to 0,25 nat, and with the dominance of genus *Hydnotrya*. In *M. glareolus* there is a strong dominance, high frequency and ecological importance of *Glomus* taxa in the first hypogeous class. In higher classes within samples from *A. flavicollis* a growing dominance, frequency and importance of *Hymenogaster* and a decline in the indexes for *Hydnotrya* can be observed. In *M. glareolus* there is a growing dominance, frequency and importance of *Rhizopogon* and a decline in dominance of *Glomus*, but still with its high frequency and ecological importance.

4.7. Morphometric analysis of spores

The size of observed spores within each genera were similar and their distributions of length and width were of normal distribution. Detailed descriptive statistics for all genera and with separation to seasons is presented in Appendix 3. Due to sample size, ANOVA I testing was used for three genera: *Hymenogaster*, *Rhizopogon* and *Glomus*, but only in case of *Rhizopogon* the results were statistically significant and have shown an increase in spore size from spring through summer and autumn (Fig. 15).

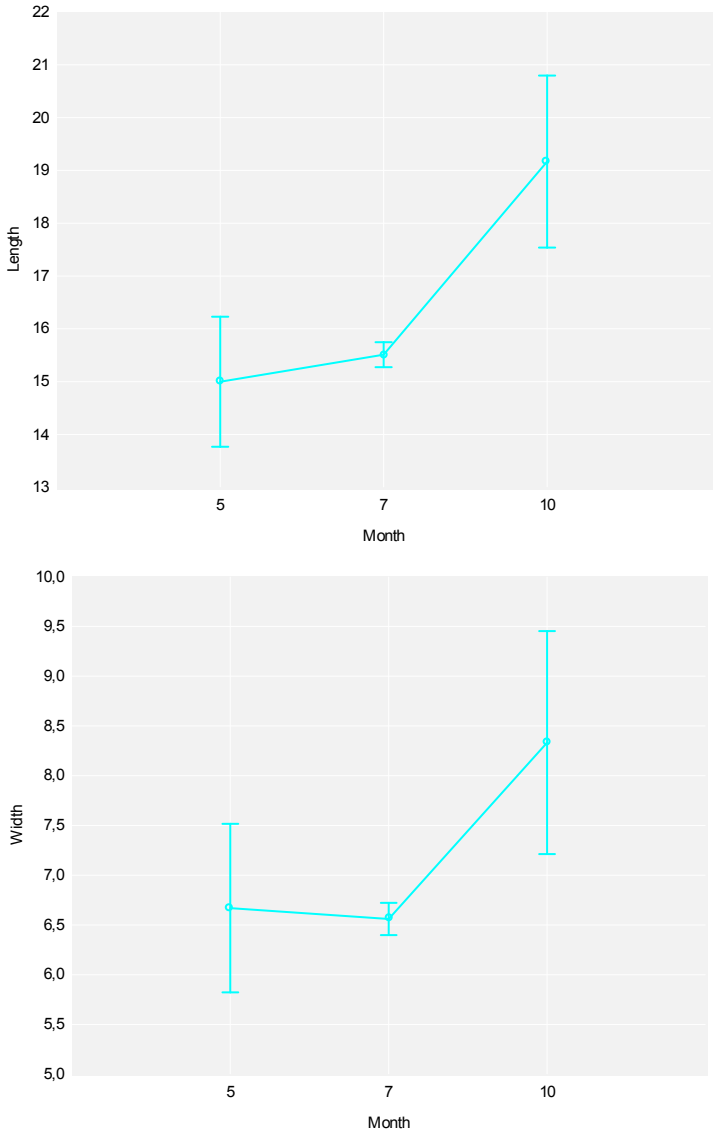


Fig. 15. Box plot showing changes in length and width of *Rhizopogon* spores in seasons (red line indicating length and blue line indicating width (Wilks' Lambda=0,87909, $F_{(6,394)}$=4,3704, p=0,00027, 95,00% CI).

5. IDENTIFIED FUNGAL TAXA

The systematics is presented according to the Catalogue of Life (www.catalogueoflife.org) and Trappe et al. 2009. The Author epithets and place of publication were taken from Index Fungorum (indexfungorum.org). Genus descriptions were prepared based on the following keys: Hawker (1954); Ławrynowicz (1979, 1988), Castellano et al. (1989), Rudnicka-Jezierska (1991), Pegler at al. (1993), Błaszkowski (2012) and other publications: Ławrynowicz (1973, 1984, 1989, 1990), Wojewoda (2003).

KINGDOM: FUNGI

Phylum: **Ascomycota**

Class: **Eurotiomycetes**

Order: **Eurotiales**

Family: **Elaphomycetaceae**

Genus: **Elaphomyces**

Class: **Pezizomycetes**

Order: **Pezizales**

Family: **Discinaceae**

Genus: **Hydnotrya**

Family: **Pezizaceae**

Genus: **Pachyphloeus**

Family: **Pyronemycetaceae**

Genus: **Genea**

Family: **Tuberaceae**

Genus: **Tube**r

Phylum: **Basidiomycota**

Class: **Agaricomycetes**

Order: **Agaricales**

Family: **Strophariaceae**

Genus: **Hymenogaster**

Order: Boletales

Family: **Paxillaceae**

Genus: **Melanogaster**

Family: **Rhizopogonaceae**

Genus: **Rhizopogon**

Family: **Sclerodermataceae**

Genus: **Scleroderma**

Order: **Gomphales**
Family: **Gomphaceae**
Genus: **Gautieria**

Phylum: **Glomeromycota**

Class: **Glomeromycetes**

Order: **Glomerales**
Family: **Glomeraceae**
Genus: **Glomus**

Phylum: **Zygomycota**

Order: **Endogonales**
Family: **Endogonaceae**
Genus: **Endogone**

5.1. Ascomycota

Elaphomyces Nees, *Syn. pl. mycet.*: 68 (1820)

Fruitbody: globose, ovoid, subglobose or irregular, 1-5 cm in diameter, tough and leathery and become brittle with age. Surface covered with warts, pyramidal and concolorous, long and sharp or low and blunt depending on the species. In some species a "crust" can be observed: a layer of soil particles and rootlets bound by hyphae. The crust's thickness depends on the type of soil and easily separated from the sporocarp. The cortex is dull-yellow, yellow-brown.

Peridium: brown, dark blue or black, smooth to ornamented with hard, rounded warts, pyramids or cones, often tomentose and enhusked by proliferated ectomycorrhizae of associated trees or shrubs, crisp-fleshy to leathery or carbonous, 2-5 mm thick. In some species it is distinctively marbled – with yellowish white veins surrounding pink do chestnut brown areas. This is the part of the sporocarp eaten by small mammals, which often discard the powdery spore mass of the gleba.

Gleba: in youth divided into sections by white and greyish pink sterile dissepiments, later becoming stuffed with cottony, spore-bearing hyphae, at maturity, the hollow is filled with a yellow to brown, olive, brownish, black, bluish black or black spore powder.

Asci and ascospores: asci globose or ellipsoid, rarely ovoid, 40-50 µm in diameter. Typically 6-8 spores in one ascus. Spores globose, yellowish-brown to brownish-black, dark brown almost black or purplish black, 20-33 µm in diameter, covered with 2-4 µm long spikes or warts. Spores found in course of this study were mostly round in shape 13,33-23,33 µm and mean diameter 19,53 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 1-3.

Odour: not distinctive to metallic or garlicy or weak and earthy.

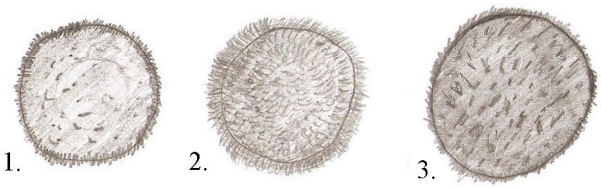
Habitat: deciduous and mixed forests, in parks, under *Quercus, Fagus, Carpinus, Betula, Corylus* trees. In coniferous forests, under Scots pine and spruce. Usually no deeper than 10 cm. In mountainous regions it reaches the edges of forest floor. Found throughout the year. In favourable conditions, the sporocarps can be present in large numbers at all times of the year. Initiation of young fruit bodies is inhibited by extreme cold or drought. Prefers fertile soils with pH 4-5.

Suspected species:

E. granulatus Fr., *Syst. mycol.* (Lundae) 3(1): 58 (1829)

E. asperulus Vittad., *Monogr. Tuberac.* (Milano): 69 (1831)

E. muricatus Fr., *Syst. mycol.* (Lundae) 3(1): 59 (1829)



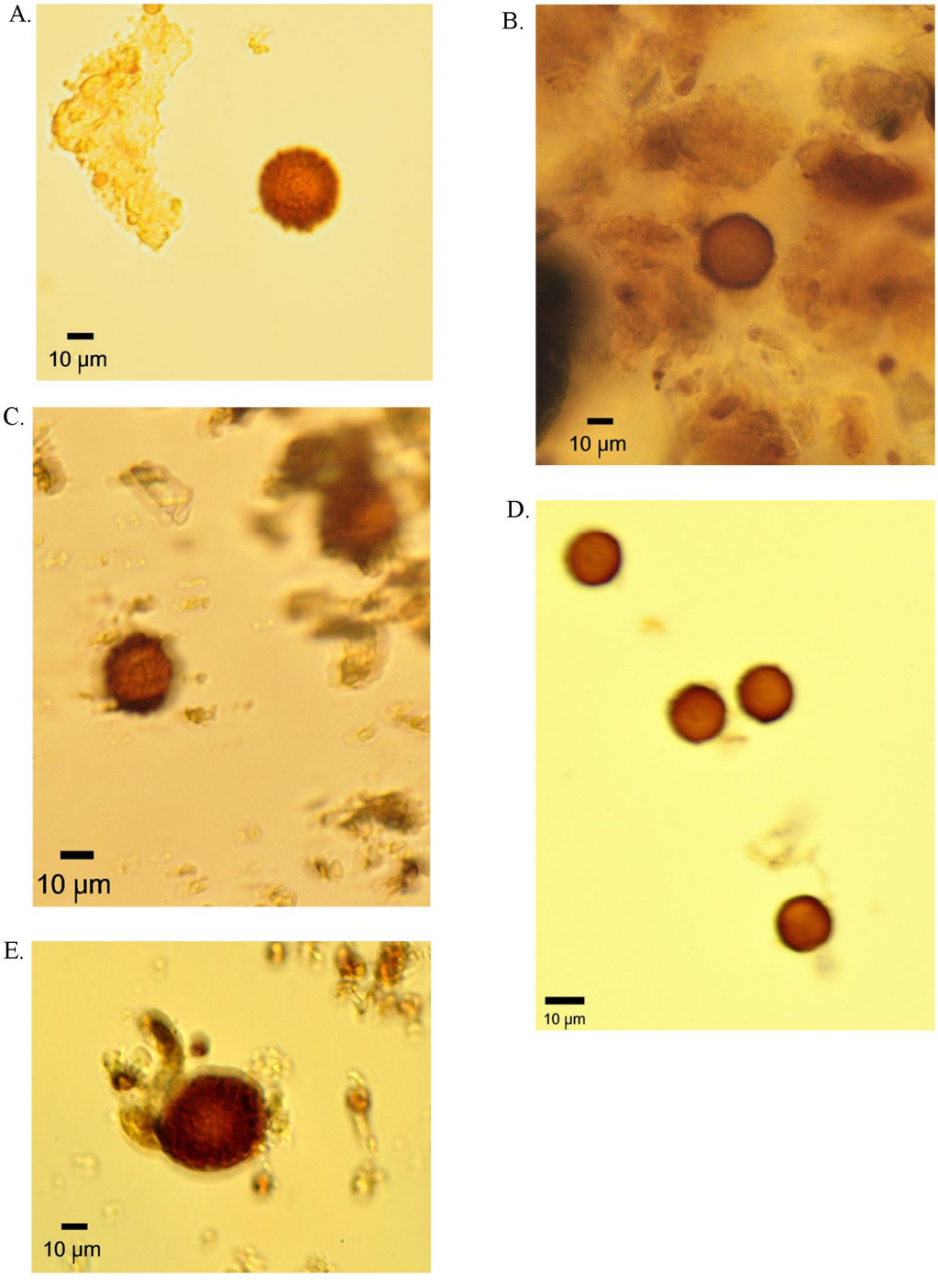


Plate 1. *Elaphomyces* spp.

1 - 3 - sketches of spores observed

A- F - photos taken under x600 magnification

Hydnotrya Berk. & Broome, *Ann. Mag. nat. Hist.*, Ser. 1 18: 78 (1846)

Fruitbody: subglobose or irregularly globose, often lobed, with wrinkled and folded surface, 0,5-6 cm in diameter. Color redish, red-brown, dark red.

Peridium: smooth or scurfy, in colour ivory to pink, orange to brown or dark purplish brown.

Gleba: hollow to fleshy-firm, divided by hollow chambers or labyrinthine canals opening to surface between folds.

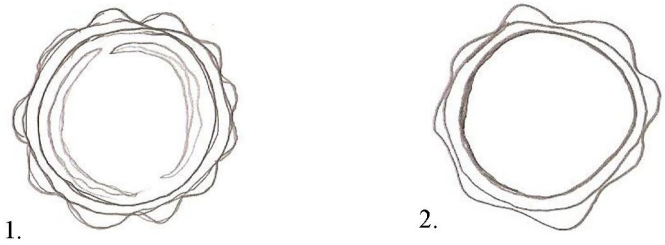
Asci and ascospores: asci long, cylindrical, clavate or long-ovoid, 150-230 µm x 35-70 µm. 6-8 spores inside. In older fruit bodies, cylindrical and uniseriate. Spores globose, 20-35 µm in diameter, with thick, deep red-brown episporium, and with rounded warts, placed irregularly. Young spores are smooth and hyaline, later becoming yellowish brown to brown, 16-34 µm x 16-35 µm without ornamentation, with a single wall 1-3 µm thick. Spores observed in the present study were mostly globose in shape 13,33-46,67 µm and mean diameter 33,97 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 4-7.

Odour: not distinctive or garlicky.

Habitat: under *Quercus, Fagus, Carpinus* trees, on packed soils with pH 4,5-5, with a good insolation, often in close proximity to roads, paths and intensely trodden places. The fruit bodies are found not deep under the soil, partly epigeically.

Suspected species:

Hydnotrya tulasnei (Berk.) Berk. & Broome, *Ann. Mag. nat. Hist.*, Ser. 1 18: 78 (1846)



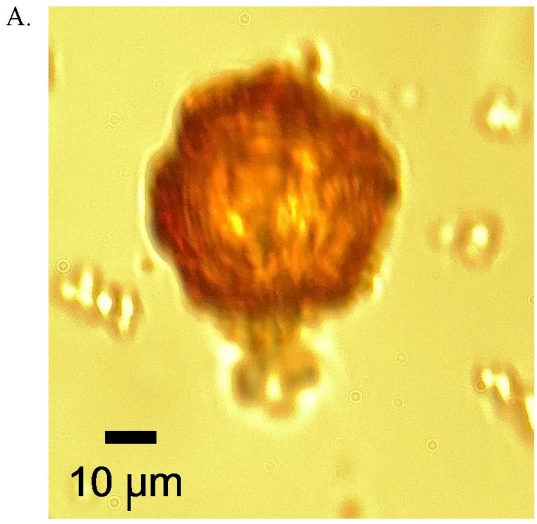


Plate 2. *Hydnotrya* sp.
1 - 2 - sketches of spores observed
A - photo taken under x600 magnification

Pachyphloeus Tul. & C. Tul., *G. bot. ital.* 1(7-8): 60 (1845), (Pachyphlodes Zobel, *Icon. fung.* (Prague) 6: 55 (1854))

Fruitbody: subglobose or globose, often with apical depression or cluster of grooves, up to 3 cm diameter, can be flattened with an apical opening and hyphae in the basis. Coloration various, black dark green, brown, yellowish-green. Surface with polygonal warts.

Peridium: brownish yellow to yellowish green, red or black, pseudoparenchymatous with reddish or violet brown cell walls.

Gleba: greyish yellow to nearly black marbled with pale veins.

Asci and ascospores: asci club shaped or cylindrical, 80-150 × 25-45 μm, 8 spores in one ascus. Spores globose 13-26 μm diameter, hyaline to light yellow-green, densely covered with 3 μm long spines or warts. During the course of this study, only two spores of *Pachyphloeus* were found, one round measuring 11,67 μm in diameter and second subglobose measuring 21,67 × 16,67 μm.

Odour: not distinctive or pungent.

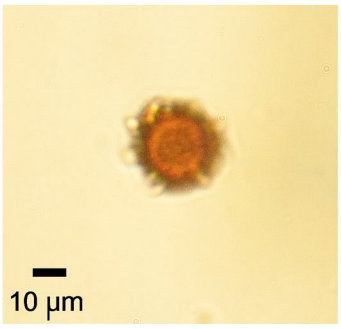
Habitat: under *Quercus, Fagus, Carpinus* trees, in exposed places. The fruit bodies lay shallow underground. Occurs from July to September.

Suspected species:

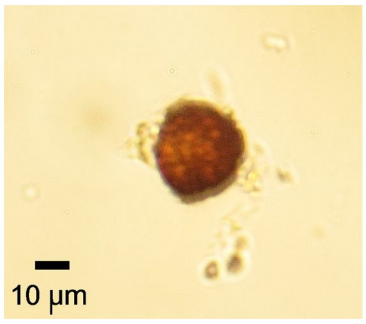
Pachyphloeus melanoxanthus (Tul. & C. Tul. ex Berk.) Tul. & C. Tul., *G. bot. ital.* 1(7-8): 69 (1845)

1.

A.



B.



C.

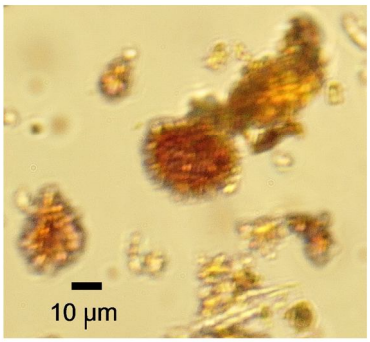


Plate 3. *Pachyphloeus* sp.

1 - sketch of a spore observed

A - C - photos taken under x600 magnification

Genea Vittad., *Monogr. Tuberac.* (Milano): 27 (1831)

Fruitbody: 0,3-3 cm in diameter, globose or irregular, with an apical opening to a hollow chamber, with a turf of hyphae in the basis of the fruit body, can be flattened on the top. Reddish brown to dark in colour.

Peridium: 4-9 cells thick, brown to black, covered in guard hairs or warty.

Gleba: white, yellow-white to yellow-brown.

Asci and ascospores: asci cylindrical, 250-300 µm × 24-35 µm, blunt on top and narrowed at the bottom, 8 spored. Spores ellipsoid, rarely almost globose, 28-42 µm × 19-28 µm, at first hyaline, smooth with lipid drops, later turning yellow to reddish. With age, episporium becomes covered with rounded or polygonal, blunt 3 × 2 µm long warts. Spores found in this study were mostly ellipsoid with the mean circularity index of 0,78. The size of spores was 16,67-31,67 × 23,33-35,00 µm and mean size was 22,30 × 28,81 µm. Detailed descriptive statistics are presented in Appendix 3, Table 8.

Odour: fungal to strongly garlicky, pungent.

Habitat: deciduous and mixed forests, most often under *Carpinus, Fagus* and *Corylus* trees and just under the surface, in the forest bed and moss layer. Occurring from July do late autumn.

Suspected species:

Genea hispidula Berk. ex Tul. & C. Tul., *Fungi hypog.*: 121 (1851)

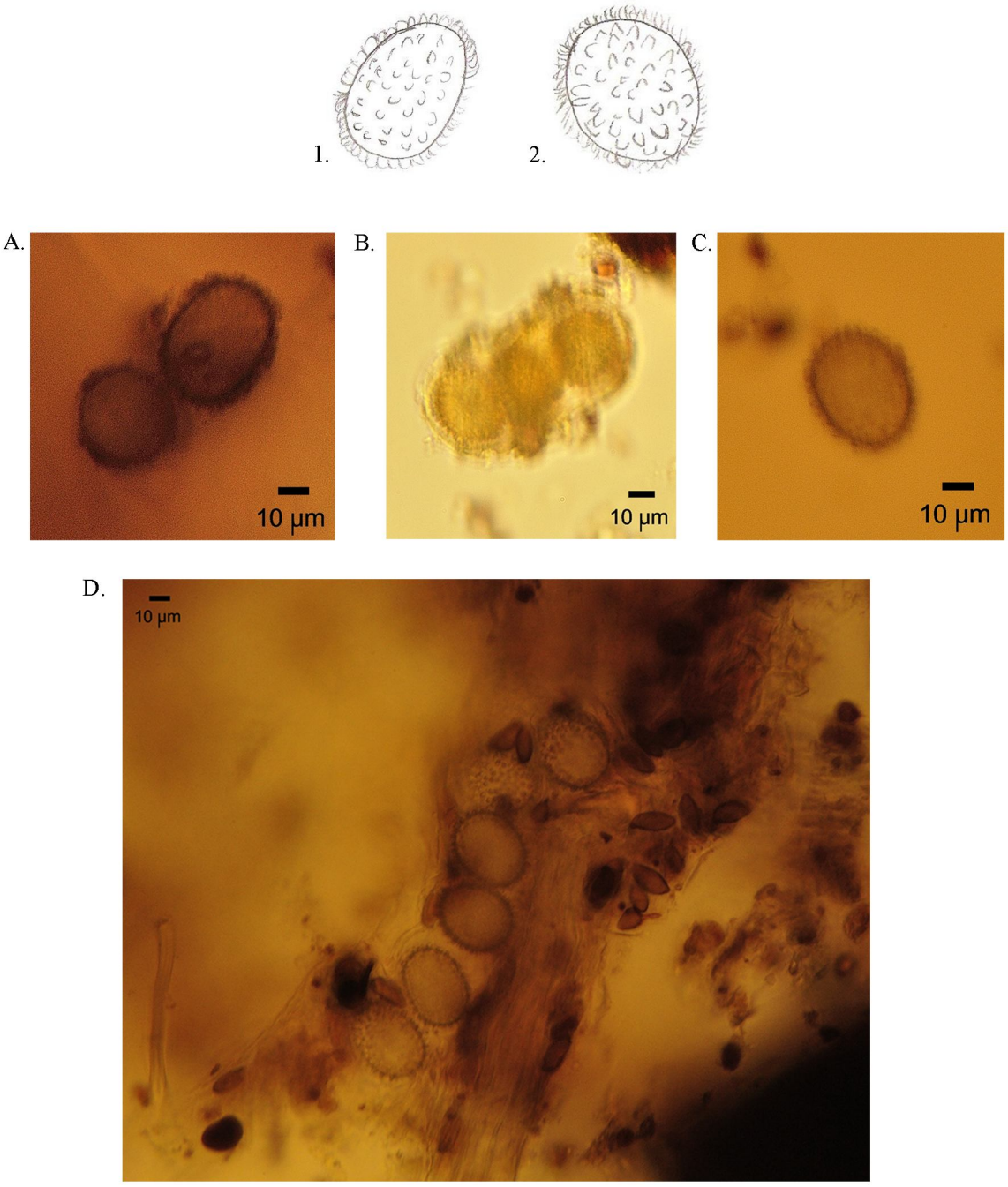


Plate 4. *Genea* sp.

1 -3 - sketches of spores observed

A - D - photos taken under x600 magnification

Tuber P. Micheli, *Nov. pl. gen.* (Florentiae): 221, table 102 (1729)

Fruitbody: globose to irregular, either firm of fleshy, 1-6 cm in diameter, various in colour. Surface can be smooth or covered with warts. Interior marbled. With age sporocarps become irregular, bumpy or lobed.

Peridium: smooth, puberulent or verrucose, and thin, yet definite. It is composed of interwoven narrow hyphae, covered in short, pointed hairs, easily separated from the interior.

Gleba: veined, white at first, later becoming fleshy pink, red-brown to purpulish black, with well developed dissepiments which later disappear with the development of asci.

Asci and ascospores: asci set on hyphae, no bigger than 150 µm diameter, globose, ellipsoid or pyriform in shape, 1-4 spores inside. Spores usually ellipsoid, sometimes globose, 17-48 µm × 12-40 µm, pale golden brown, light yellow to brown and deep red-brown when mature. Wall of the spores is covered with spines or reticulum. Spores observed in this study were mostly ellipsoid with the mean circularity index of 0,79. The size of spores was 13,33-40,00 × 16,67-48,33 µm and mean size was 20,21 × 25,76 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 9-11.

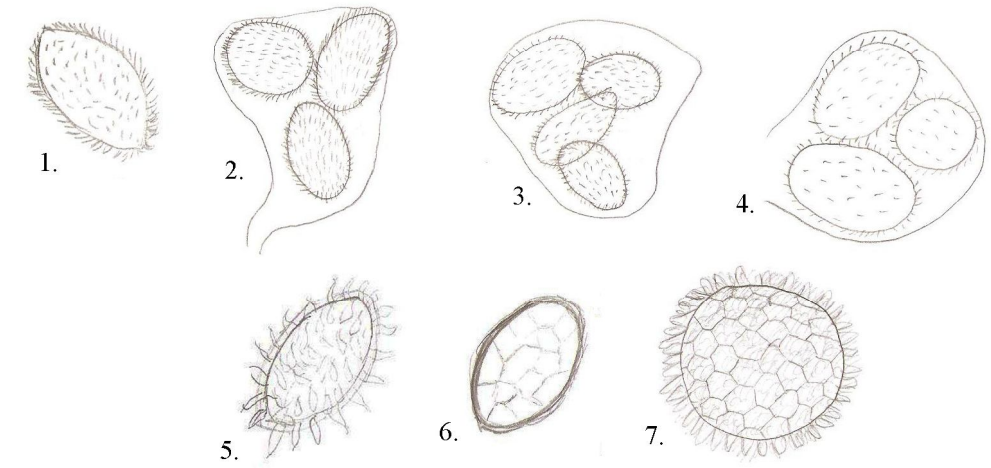
Odour: usually prominent, pungent, garlicky, cheesy or wine-like.

Habitat: occurs in various types of forests as well as grassy terrain far from trees, under *Quercus*, *Crataegus*, *Tilia* and *Larix* trees, can be found from June to October. Occurs in the humus layer of light and fertile soils, in places with bare soil with removed litter cover, near roads, footpaths or under the litter cover and even in deeper layers.

Suspected species:

T. rufum Pico, *Meleth. bot.*: 80 (1788)

T. puberulum Berk. & Broome, *Ann. Mag. nat. Hist.*, Ser. 1 18: 81 (1846)



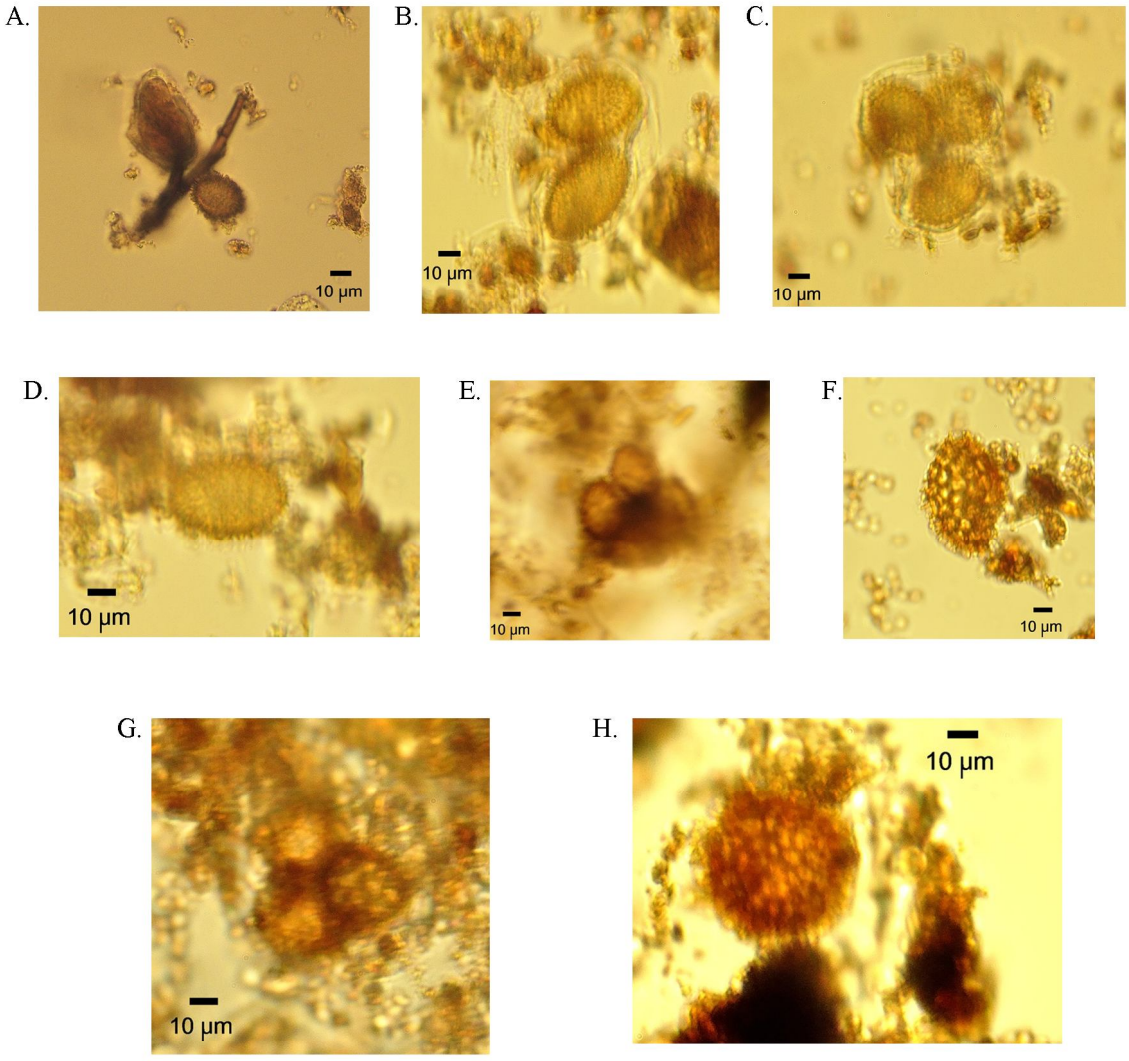


Plate 5. *Tuber* sp.

1 - 5 - sketches of observed spores and asci of *T. rufum*

6 - 7 - sketches of observed spores of *T. puberulum*

A - D - photos of *T. rufum* taken under x600 magnification

E - H - photos of *T. puberulum* taken under x600 magnification

5.2. Basidiomycota

Hymenogaster Vittad., *Monogr. Tuberac.* (Milano): 30 (1831)

Fruitbody: globose, subglobose to irregular, 0,4-15 cm in diameter, at first white, later becoming dingy white, sometimes reddish when bruised.

Peridium: smooth, thin in fragile, white to gray, olive, dull yellow, yellowish-brown to blackish brown, in some species staining lilac to blue or black when exposed, at first soft but brittle and tends to split in older specimens.

Gleba: cinnamon to dark brown, occasionally with a few sterile veins arising from the basal pad, at first white, then lilac to mushroom pink, grey or greyish brown in mature fruitbodies.

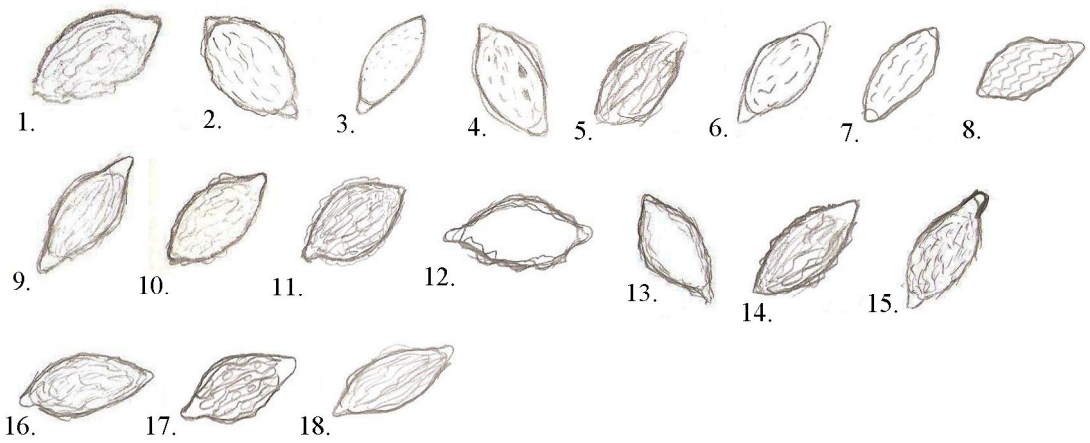
Basidia and basidiospores: basidia about 11 µm × 19 µm, 1-4 spored, broadly clavate in shape, sterigmata short, conical. Spores wrinkled, can be ornamented with warts or pegs or irregularly ridged. In shape spores are longitudinally symmetrical, ellipsoid to ovoid, citriform or subcylindric, and in most species with an obscure or prominent apical projection. The size of the spores varies: 9-35 µm × 4,5-18 µm with ornamentation. Spores observed in this study were ornamented, longitudinally symmetrical and ellipsoid with the mean circularity index of 0,56. The size of spores was 6,67-20,00 × 10,00-33,33 µm and mean size was 10,19 × 18,55 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 12-15.

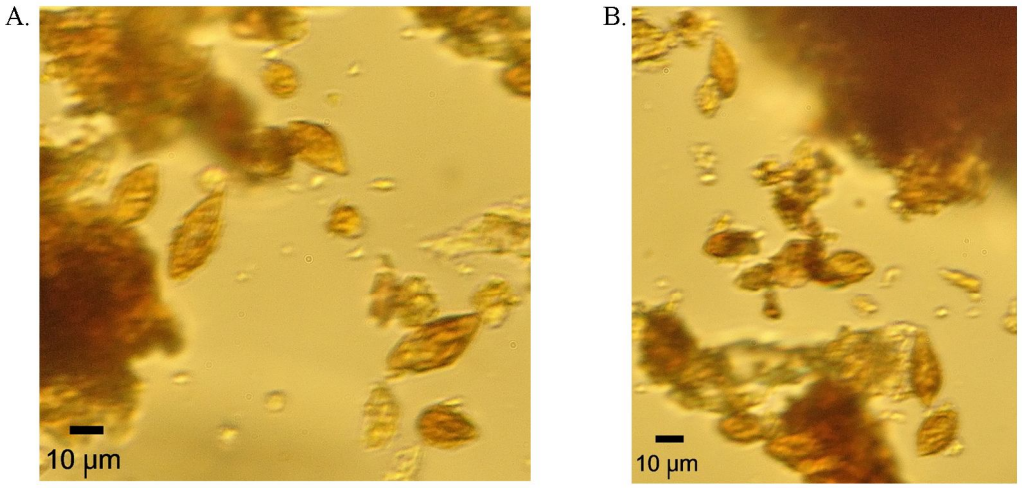
Odour: characteristic, sometimes unpleasant.

Habitat: occurs in groups on well developed, branched rhizomorphs below loose layer of leaf litter and humus, no more than 5 cm in calcareous areas and throughout the year if conditions are favourable (Pegler et al. 1993). The species occurs under *Quercus robur* and *Tilia cordata* trees.

Suspected species:

H. tener Berk., *Ann. Mag. nat. Hist.*, Ser. 1 13: 349 (1844)





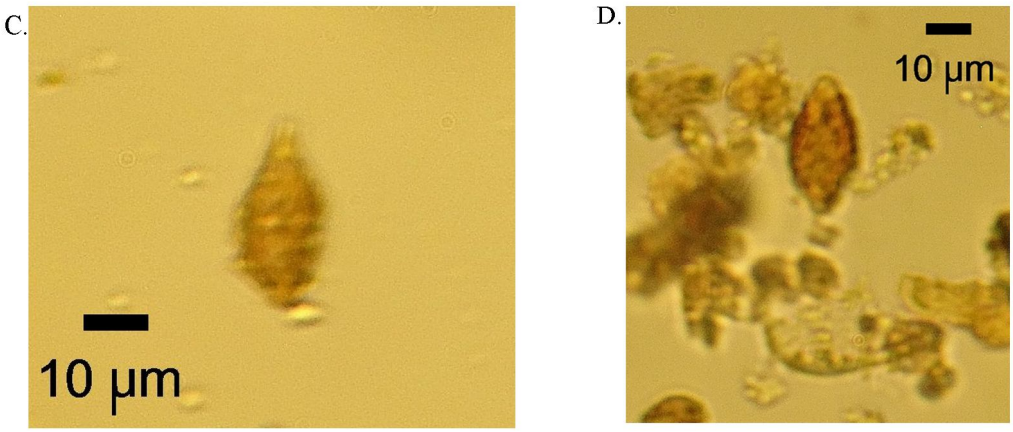


Plate 6. *Hymenogaster* sp.

1 - 18 - sketches of spores observed

A - D - photos taken under x600 maginification

Melanogaster Corda, in Sturm, *Deutschl. Fl.*, 3 Abt. (Pilze Deutschl.) 3(11): 1 (1831)

Fruitbody: more or less globose or ellipsoidal, irregularly globose to lobed, sometimes confluent,, with size 0,5-4 cm in diameter. Coloration is at first dull reddish brown and becomes potato-colored or even olive-brown, blotched black when bruised.

Peridium: tomentose, dissepiments thick and cavities large and filled with gelatinous matrix in which basidia develop irregularly. At first ochraceous, pale greenish-yellow to mustard yellow and latter becomes fuscous brown with a reddish tint to primrose tint.

Gleba: Gleba is white, pale yellow at first with blue-black chambers separated by white or dingy white dissepiments, later on reddish fuscous to purplish black when mature. Chambers are willed with gelatinous contens of a black slimy mass of spores.

Basidia and basidiospores: Basidia are clavate, 20-35 µm × 5-8 µm, tapering to base, 2-4 spores borne on a slender 1-6 µm long sterigmata. Spores are usually dark colored, fuscus brown or olive-brown, spindle-shaped, ellipsoid or cylindrical with a pointed apex and a clow-like process at the base. Spores observed in this study were spindle-shaped and with the characteristic pointed apex. The mean circularity index was 0,57. The size of spores was 6,67-15,00 × 10,00-28,33 µm and mean size was 8,28 × 14,81 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 16-18.

Odour: at first sweetish, slight, pleasant and fruity or mildly unpleasant, rubbery, strong and foetid.

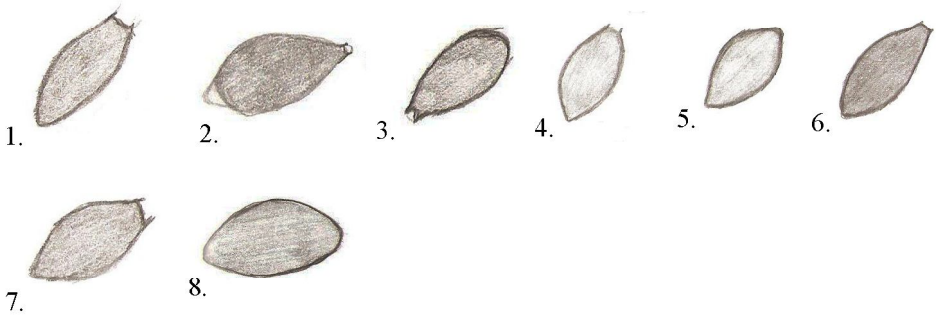
Habitat: occurs throughout the year in humus, under loose litter, near the soil surface, or even on the surface, usually under *Fagus* and sometimes other trees, *Abies alba* and *Carpinus betulus*.

Suspected species:

M. ambiguous (Vittad.) Tul. & C. Tul., *Annls Sci. Nat.*, Bot., sér. 2 19: 378 (1843)

M. variegatus (Vittad.) Tul. & C. Tul., *Fungi hypog.*: 92 (1851),

M. broomeianus Berk. [as '*broomeianus*'], *Ann. Mag. nat. Hist.*, Ser. 1 10: 377 (1843) [1842]



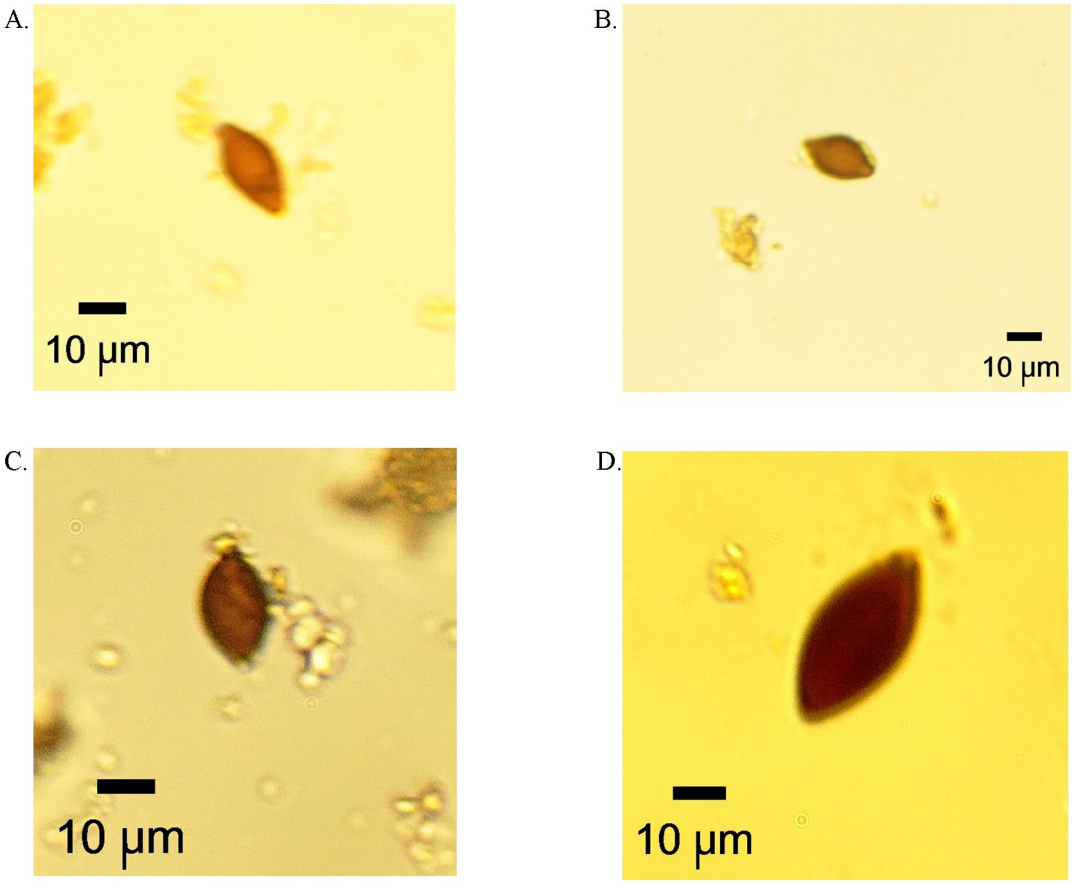


Plate 7. *Melanogaster* spp.

1 - 8 - sketches of spores observed

A - D - photos taken under x600 magnification

Rhizopogon Fr., in Fries & Nordholm, *Symb. gasteromyc.* (Lund) 1: 5 (1817)

Fruitbody: 1-6 cm in diameter, globose to pyriform to irregular. Some species reach the size up to 15 cm in diameter.

Peridium: white to brownish, reddish brown or red, often with the top darker than the base and ranging in thickness from 0,5-2 mm. In many species the peridium is stained pink to brick red or brown but in some it is smooth or felty. Most of the species have rhizomorphs appressed around the base and sides.

Gleba: white or yellow at first, with small, empty chambers. In some species the chamber is filled with spore powder. At maturity the gleba becomes olive, olive gray, olive brown, orange brown or blackish brown.

Odour: depends on the species, can be fruity, wine like, cheesy or spicy-pungent.

Basidia and basidiospores: basidia lageniform, with a ventricose base, bearing 4-8 spores. Spores are in most species smooth, longitudinally symethrical and cylindric to fusoid. Some species have ellipsoid or irregular spores. The size of the spores ranges from 5-15 (max 20) × 1,5-8 µm. The spores have a straight attachment, an inconspicuous nipple or a basal, cupped truncation if the spore. Spores observed in this study were smooth, longitudinally symmetrical and cylindric with the mean circularity index of 0,43. The size of spores was 5,00-16,67 × 10,00-20,00 µm and mean size was 6,60 × 15,57 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 19-22.

Odour: not distinctive.

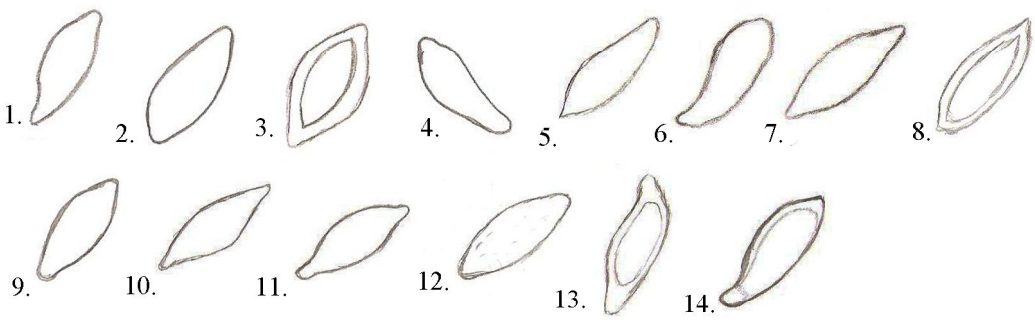
Habitat: Occurs mostly on sandy soils in coniferous and mixed forests and pine forests, in sunny, xerorhermic places and sand dunes, from spring to autumn.

Suspected species:

R. nigriscens Coker & Couch, *Gasteromycetes E. U.S. Canada* (Chapel Hill): 30 (1928)

R. obtextus (Spreng.) R. Rauschert, in Hirsch, *Wiss. Z. Friedrich Schiller-Univ. Jena*, Math.-nat. Reihe 33(6): 818 (1984)

R. roseolus (Corda) Th. Fr., *Svensk bot. Tidskr.* 3: 282 (1909)



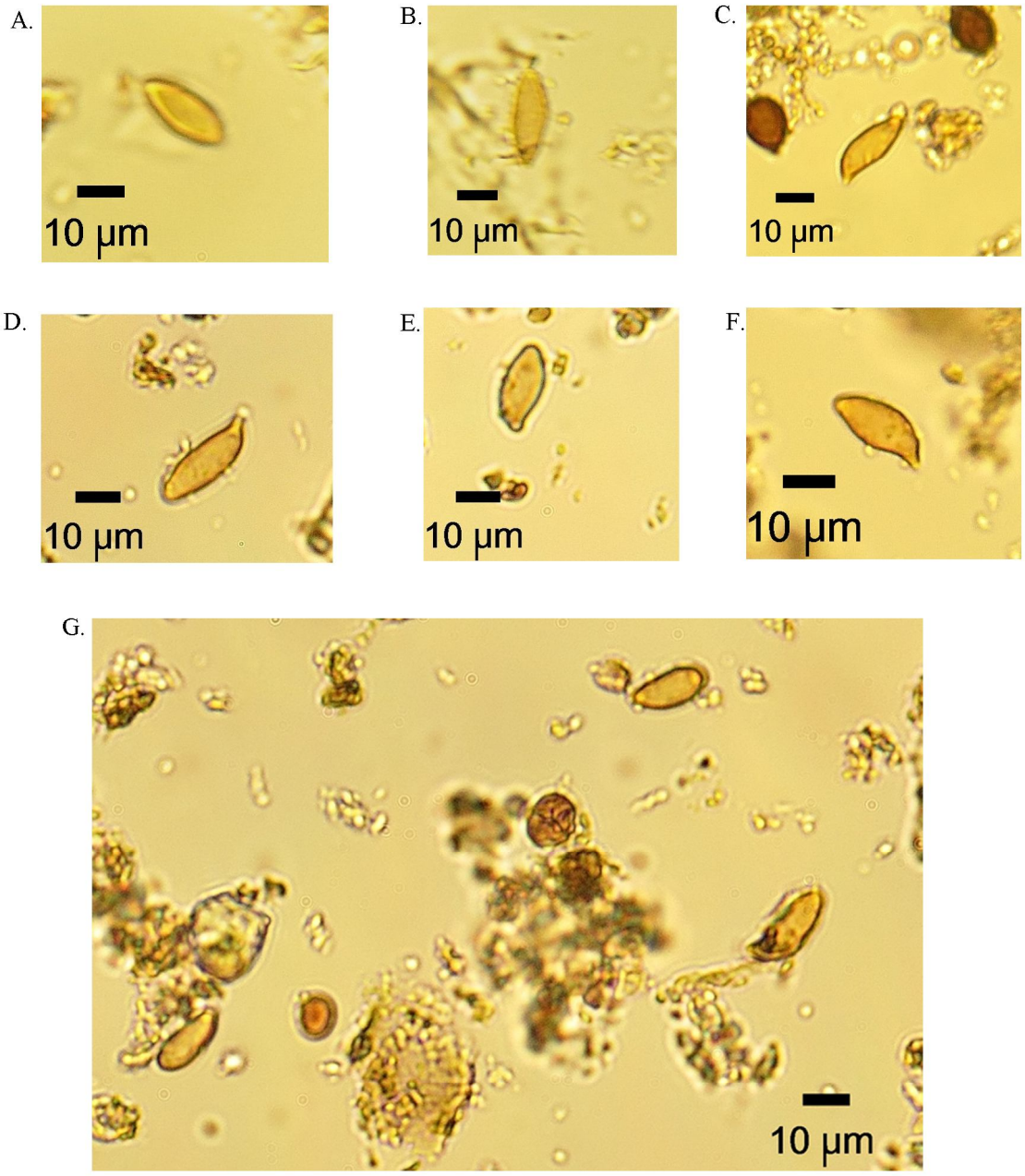


Plate 8. *Rhizopogon* spp.

1 - 14 - sketches of spores observed

A - G - photos taken under x600 magnification

Scleroderma Pers., *Syn. meth. fung.* (Göttingen) 1: xiv, 150 (1801)

Fruitbody: subglobose to irregulat, 2-6 cm in diameter, with a cluster of rhizomorphs at the base, covered in shells or warts, cracking in small fields. Usually more or less yellowish or brownish.

Peridium: pale brown or pale yellow to brownish yellow, becoming rosy blush when bruised and pink when cut, smooth or slightly scaly, 3-6 mm thick.

Gleba: White and solid, at maturity olivaceous black to purpulish black.

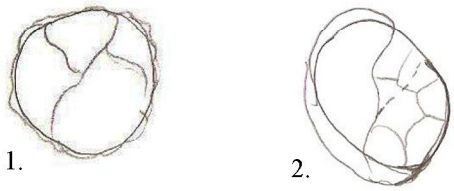
Basidia and basidiospores: basidia roughly clavate 4-8 spored. Spores globose, 7-15 µm in diameter, covered in spikes, and with a reticulum on surface. Spores found this study were mostly round in shape 8,33-20,00 µm and mean diameter 14,58 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 23, 24.

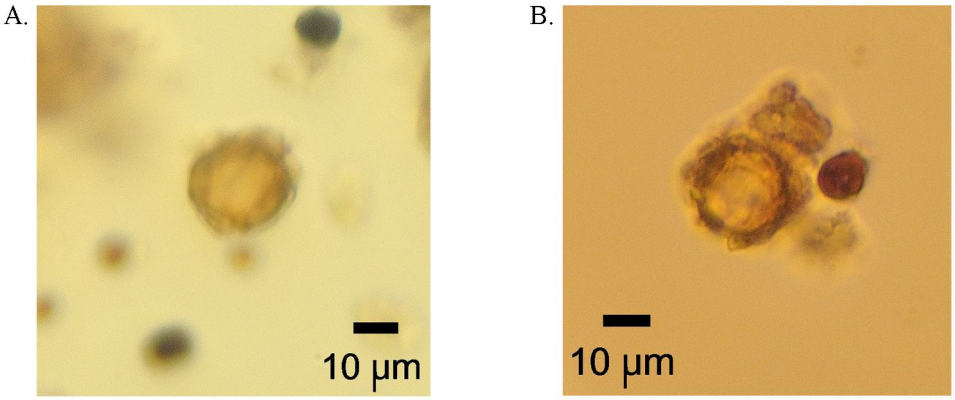
Odour: strong, aromatic.

Habitat: epigeous, or sometimes embedded in the ground, mostly occurring on sandy soils, in coniferous and mixed forests, at the forest edges, near walk ways, also on tree trunks. Occurs in summer and autumn.

Suspected species:

S. citrinum Pers., *Syn. meth. fung.* (Göttingen) 1: 153 (1801)





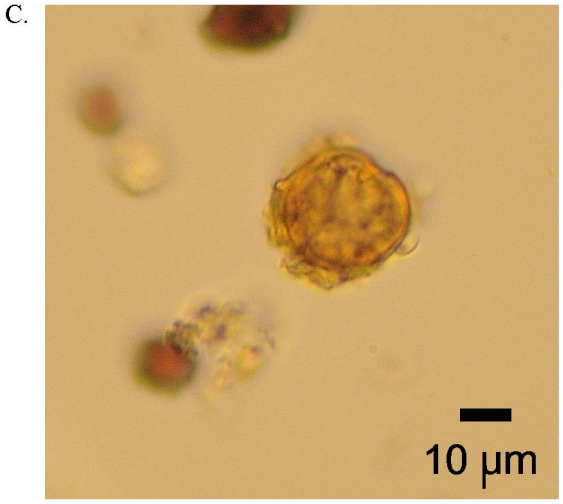


Plate 9. *Scleroderma* sp.

1 - 2 0 sketches of spores observed

A - C - photos taken under x600 magnification

Gautieria Vittad., *Monogr. Tuberac.* (Milano): 25 (1831)

Fruitbody: 1-8 cm broad, globose, subglobose or irregular, usually with a permanent rhizomorphs at the base.

Peridium: fragile and ephemeral in most species, dingy white to brown.

Gleba: cinnamon to dark brownish black, consists small or prominent, labirynthine chambers and a poorly or strongly developed cartilaginous columella.

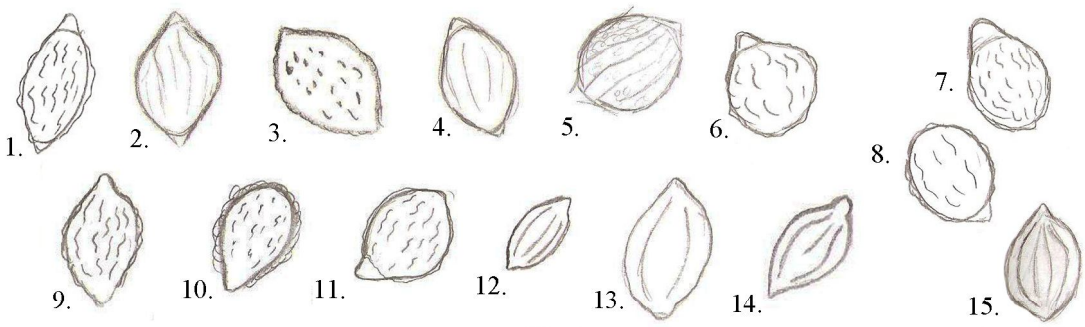
Basidia and basidiospores: basidia cylindrico-clavate, 1-4 spored. Spores longitudinally symmetrical, ellipsoid, ovoid or globose, 10-32 × 6-18 µm including ornamentation. Ornamentation with longitudinal, slightly piraled and forked ridges. Spores observed in this study were ornamented, longitudinally symmetrical and ellipsoid to citriform The mean circularity index was 0,61. The size of spores was 6,67-15,00 × 11,67-21,67 µm and mean size was 9,94 × 16,50 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 25-27.

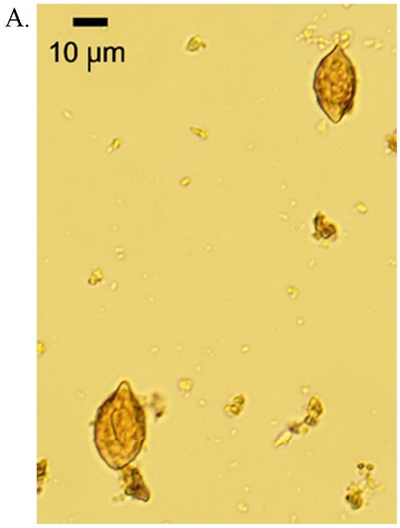
Odour: not distinctive, mushroom-like in young fruit bodies, often becoming intensive, nauseous sweet-oily to sewer-gaseous.

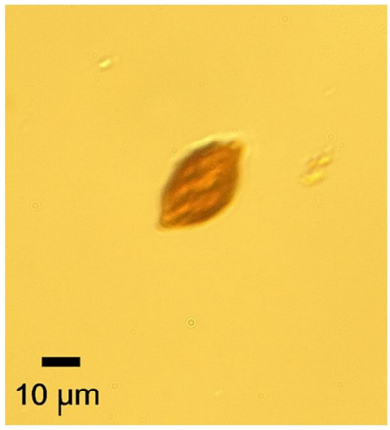
Habitat: occurs throughout the year, under deciduous trees mostly in early spring, even under melting snow.

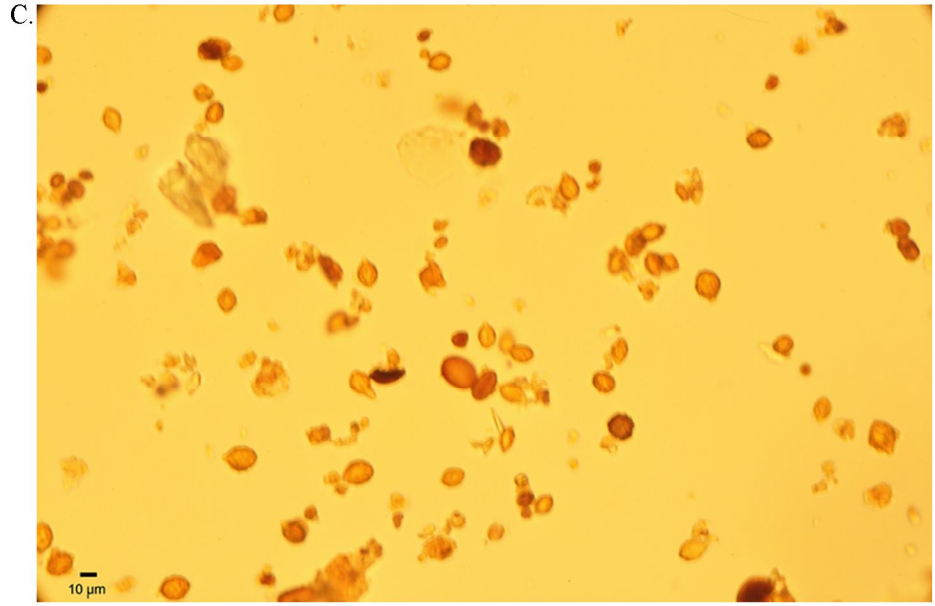
Suspected species:

G. morchelliformis Vittad., *Monogr. Tuberac.* (Milano): 26 (1831)









Palte 10. *Gautieria* sp.

1 - 15 - sketches of spores observed

A - C - photos taken under x600 magnification

5.3. Glomeromycota

Glomus Tul. & C. Tul., *G. bot. ital.* 1(2): 63 (1844)

Fruitbody: globose to convoluted or irregular, 1-10 mm broad.

Peridium: when present white to yellow or brown, smooth or cottony, otherwise absent.

Gleba: white to yellow, brown, nearly black.

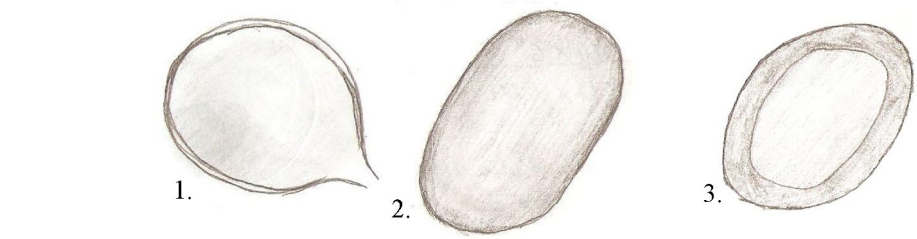
Chlamydospores: placed randomly in the gleba or align in rows radiating from the base, globose to ellipsoid or pyriform, 20-310 × 18-305 µm when smooth, when ornamented 105-452 × 169-470 µm (without ornamentation), thick 3-layered wall, 2-18 µm. Spores observed in this study were round or ellipsoid in shape with size ranging from 6,00-21,67 µm to 143,33-166,67 µm. The mean circularity index was 0,91 and the mean size 55,48-61,78 µm. Most spores had a prominent, thick outer wall. Detailed descriptive statistics are presented in Appendix 3, Tables 28-31.

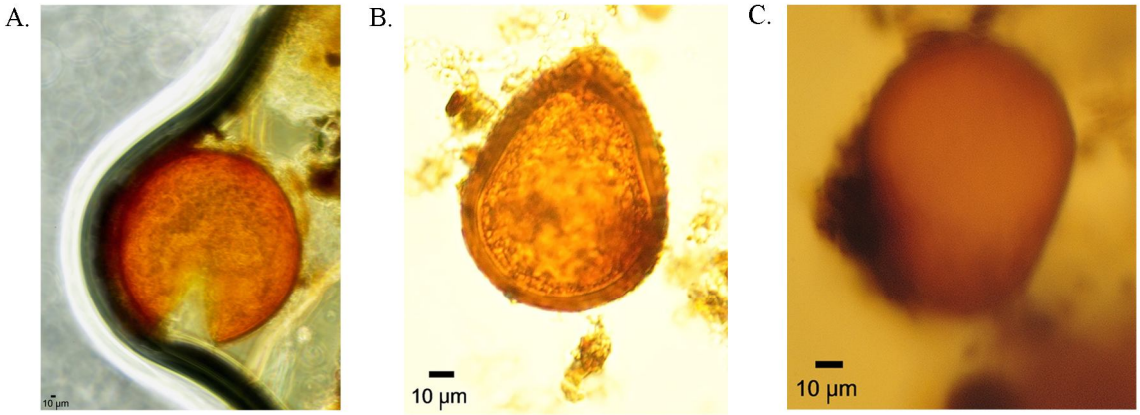
Odour: not distinctive.

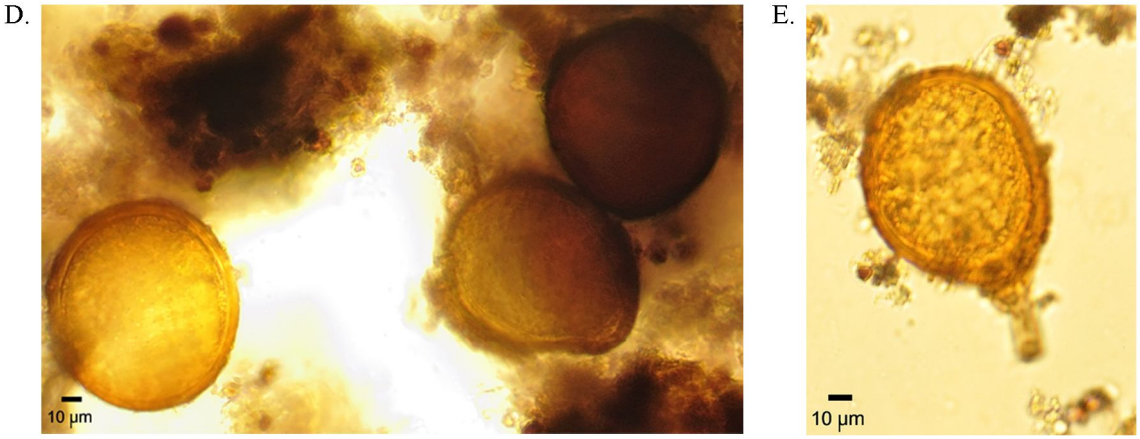
Habitat: found throughout the year in various forests under deciduous trees.

Suspected species:

G. macrocarpum Tul. & C. Tul. [as 'macrocarpus'], *G. bot. ital.* 1(2): 63 (1844)







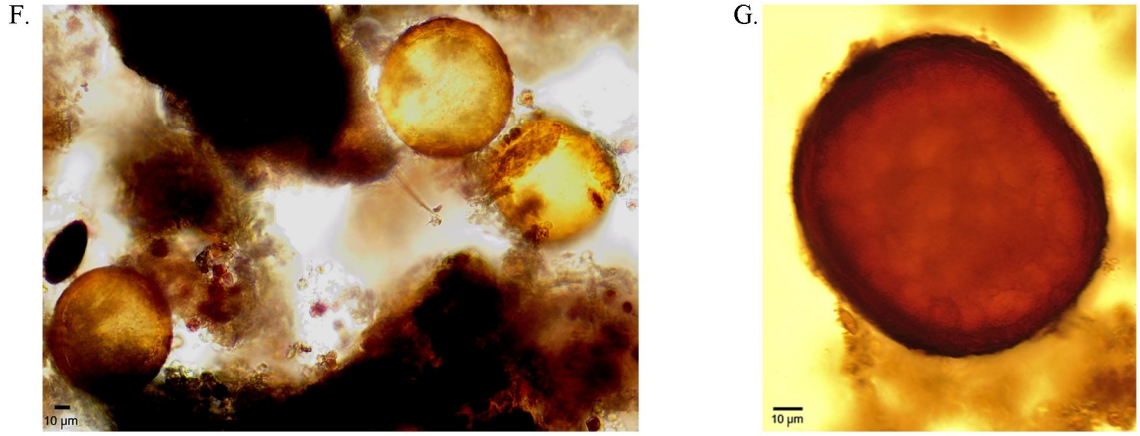


Plate 11. *Glomus* sp.

1 - 3 - sketches of spores observed

A - G - photos taken under x600 magnification

5.4. Zygomycota

Endogone Link, *Mag. Gesell. naturf. Freunde, Berlin* 3(1-2): 33 (1809)

Fruitbody: in present, subglobose to irregular, 1-2,5 cm in diameter, white or white with yellow, grey or pink shade. Some species secrete milk when cut through.

Peridium: when present white to yellow or brown, smooth or cottony, otherwise absent.

Gleba: grey to bright yellow or brown, loose in structure.

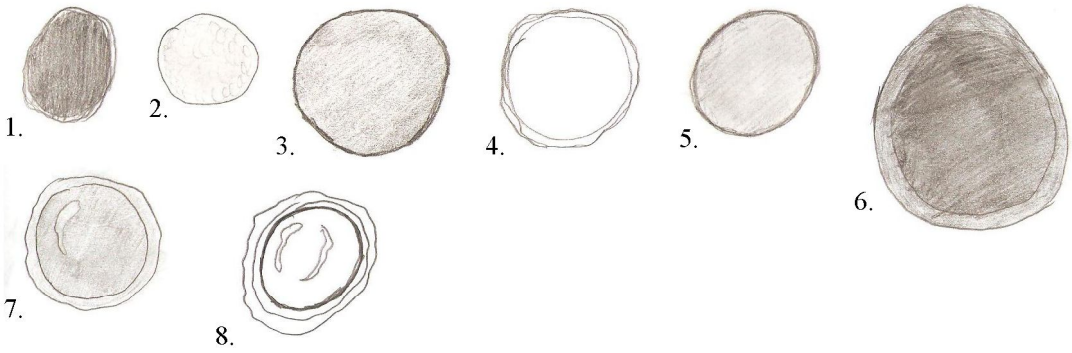
Zygospore: ellipsoid to globose or irregular, when smooth 27-150 × 27-120 µm, when mantled by abherent hyphae 52-150 × 41-190 µm (without the hyphae), 2-layered wall, 2-11 µm. Spores observed in this study were round or ellipsoid in shape with size raging from 20,83 × 21,67 µm to 68,33 × 73,33 µm. The mean circularity index was 0,88 and the mean size 40,96-47,44 µm. Detailed descriptive statistics are presented in Appendix 3, Tables 32-34.

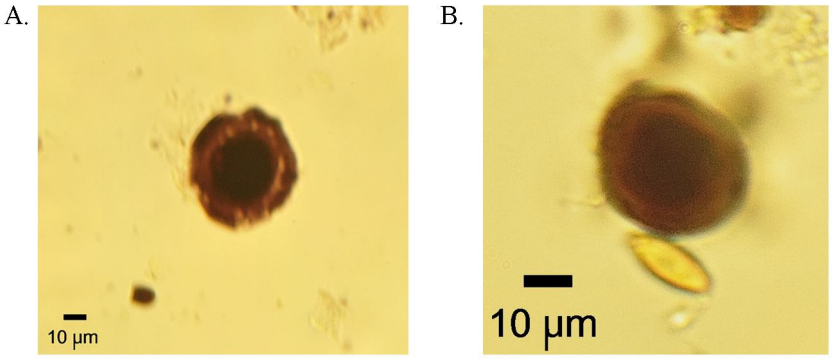
Odour: not distinctive to garlicky.

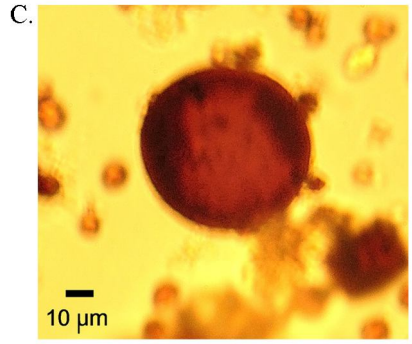
Habitat: occurs commonly and throughout the year in coniferous and mixed forests, saprobic, or ectomycorrhizal with *Fagus, Quercus, Larix, Picea, Pinus* and *Taxus*. Found the surface layers of the soil, underneath leaves, mosses and ceased wood.

Suspected species:

E. lactiflua Berk., Ann. *Mag. nat. Hist., Ser*. 1 18: 81 (1846)







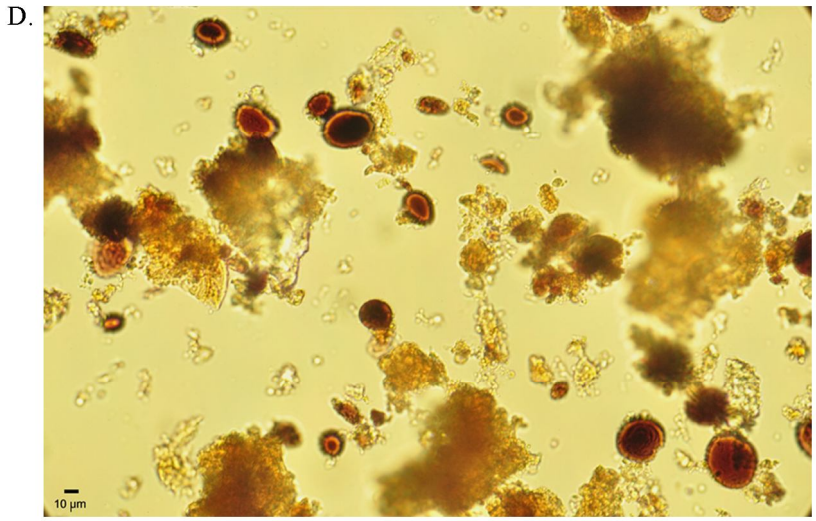


Plate 12. *Endogone* sp.
1 - 8 - sketches od spores observed
A - D - photoes taken under x600 magnification

6. DISCUSSION

6.1. Differences in spore number between years, study area and seasons

The results of this study, despite the large number of zero attempts, show overall trends in mycophagy and its dependence on the environment, fungal availability, and accessibility of other food items.

Difference in spore numbers between years has shown a significance in basidiomycetes from summer samples only. The testing has shown a significant relation between number of spores found in faecal samples and the mean temperature of the given month. Results of GLM analysis have shown that the mean temperature and rainfall combined with wind had the greatest impact on the mean spore number. If that number in samples is an indicator of sporocarp availability in the habitat (as suggested by a number of studies, eg. Claridge, Lindenmayer 1998; Kataržytė, Kutorga 2011; Remick 2015), then this means fungi produce fruit bodies in high temperatures, and with a mild amount of rainfall (when there are no storms). This corresponds to well documented observations (Fogel 1976; Ure, Maser 1982; Luoma et al. 2003). Hypogeous fungi (especially the genus *Tuber*) depend on fruiting triggers such as sunshine hours, summer rainfall and summer temperatures. The mycelium can be present in the environment, but the fungus will not fruit when the temperature and rainfall are not met (Thomas 2014).

Difference between the years occur depending on the study area (Bertolino et al. 2004). In Spała more traps were in close distance to the road, where significantly more spores were found. Many hypogeous genera fruit in close proximity to pathways and ground roads where there is less plant vegetation (Ławrynowicz 1988, 2009). The difference between the study plots in Spała and Konewka indicates a higher fungal diversity in Spała. Mean numbers for all spore groups were connected with the study area, with ascomycetes having the strongest interaction. The results for spores of basidio- and glomeromycetes were not as statistically significant, as the influence of weather conditions. The main difference between the two study plots is that Spała is closer to Pilica river, the terrain is generally lower in altitude and the microclimate is more humid. This suggests that hypogeous ascomycetes are more dependent on local

conditions than the other groups. In the study of Kataržytė and Kutorga (2011) seasonal mycophagy also mirrored the availability of sporocarps in the study area.

It is generally observed that ingestion of fungal material by animals varies between seasons (Kataržytė, Kutorga 2011; Schickmann et al. 2012). In this study, the greatest number of spores was found in samples taken in summer although in most studies, the largest amounts of hypogeous spores were found in samples taken in autumn, or that summer and autumn results were similar (Claridge, Lindenmayer 1998; Bertolino et al. 2004; Schickmann et al. 2012). For example, Kataržytė and Kutorga (2011) found most hypogeous fungi were eaten in autumn and least in spring, when there was only *Elaphomyces* detected in faecal samples. On the other hand, Ovasaka and Herman (1986) in their studies found highest amounts of hypogeous fungi in animal samples in summer and noted a decline in mycophagy in dry summers. McKeever (1960) in his paper demonstrated that flying squirrels *Glaucomys sabrinus* consume lichenized fungi in winter when the snow cover is deep. During spring, as the snow cover depletes, squirrels consume some amount of hypogeous fungal material and in summer it dominates in their diet. This however may also depend on animal species. Remick (2015) in his study compared seasonal changes in mycophagy of red-back voles *Myodes gapperi* with *Tamias* chipmunks. He found that *M. gapperi* consumed fungi in a stable fashion throughout summer, but the *Tamias* chipmunks eat increasingly more fungi during summer.

Seasonal changes in mammalian mycophagy depend not only on the availability of sporocarps in the habitat, but also on the accessibility of other food sources (Claridge 2002). In this study, both *A. flavicollis* and *M. glareolus* ate more fungal material in summer and autumn (both hypo- and epigeous). Both species are occasional mycophages, consuming fungi while foraging on different kind of food, or when the main food source is unavailable. In north-eastern Europe, the main food source for the *A. flavicollis* are grain, green parts of plants and invertebrates. In contrary, *M. glareolus* favours green parts of plants, seeds, fruits and invertebrates (Kataržytė, Kutorga 2011). *M. glareolus* is generally regarded as more mycophagous than *A. flavicollis* (Kataržytė, Kutorga 2011; Schickmann et al. 2012).

6.2. Differences between species, age groups and sexes

Results of this study show that, in comparison, significantly higher mean number of spores was found in samples from *M. glareolus* than from *A. flavicollis*. The explanation might be that vole corridors and dens are approximately at the same depth as the fruiting zone of hypogeous fungi, what makes it easy for the animals to come across fruit bodies. In most studies, including this one, it is confirmed that vole diet is more diverse in fungal material. In comparison *A. flavicollis* is more active above the ground, foraging on vegetation in the understory and even in tree brunches. However mice build their nest underground and between tree roots. This explains why in both species young individuals ate fungi in greater quantities, as the young stay in the nests more than adults and are later more likely to forage closer to the ground. During field work and live trappings, cases of two or even three animals caught in one live trap were to be observed quite frequently. Most animals caught together ranged from 14,0 to 26,0 grams and were similar in weight. This indicates that young animals forage together, during the stage of separation from their mother, and some keep together for a longer period.

Females of *A. flavicollis* spend more time in the nests when they bring up their young. This corresponds to females of this species consuming more hypogeous fungi in relation to males. Such difference does not occur in bank voles, as both males and females live and forage under or close to the ground. Bertolino et al. (2004) in their study of red squirrels *Sciurus vulgaris*, found that both males and females are equally likely to consume fungi. No differences between sexes were also noted in flying squirrels *G. sabrinus* (Maser et al. 1985). However, McIntire (1984) found that female *Eutamias* chipmunks consume hypogeous fungi to a higher extend. Female chipmunks show a higher orientation towards stumps, logs and woody debris than males (Walker 1923; Brand 1974; States 1976) and such habitats are also fruiting places for hypogeous fungi. Therefore differences between male and female diet are present when there is a difference in foraging strategy, which can seasonally enhance fungal consumption.

Animals can find hypogeous fungi with ease, and as dispersion vector they are more reliable than wind (Claridge 2002; Jacobs, Luoma 2008). It however would not be the case of the distance, as young voles do not disperse very much if the conditions in the habitat they were born in are good and the population size is optimal. They will disperse greatly when the conditions worsen or their population size is too big (Sokolov

1981). Moreover, they will carry the spores where they forage - into rooting zones of potential mycorrhizal host plants (Maser et al. 1978a, 1988; Claridge et al. 1992; Johnson 1994, 1996; Trappe, Claridge 2005; Trappe et al. 2009). While foraging on hypogeous fungi, the animals carry spores not only in their stomachs, but also on their fur and claws, leaving the spores as they dig for food and pass through the rooting zone (Maser et al. 1988; Claridge et al. 1992). To form mycorrhiza the number of spores in the deposited faecal material must be sufficient. Overtime spores may migrate deeper into the soil with rain water and accumulate in the rooting zone. Animal faeces provide a better inoculum than wind dispersion, as they form a concentrated mass of diverse spores. By this, animals ensure the genetic and species diversity of the fungal community and promote formation of mycorrhiza (McIlveen et al. 1976; Maser et al. 1978a; Claridge et al. 1992; Trappe, Claridge 2005). Mammal dispersion enhances mycorrhizal inoculation, but not as sufficient as inoculation with already developed hyphae from mature trees (Caldwell et al. 2005). Although the mature forests are the richest in mycorrhizal inoculation material, rodents disperse mycorrhizal inoculum into new networks on the edge of ecosystems (Maser et al. 1978a; Pyare, Longland 2001; Frank et al. 2009). For example, *G. sabrinus* habitat covers not only the middle of the forest, but also forest edge and clearcuts. This makes the squirrel a good spore vector into new habitats (Flaherty et al. 2008). Animals also carry spores into early succession habitats, like glacier forefronts and burnt down forest patches (Cázares, Trappe 1994; Pyare, Longland 2001).

6.3. Foraging strategies in mycophagous animals

Analysis of the Shanon's diversity index distribution shows how mycophagous the animals are (Jacobs, Luoma 2008). Samples from *M. glareolus* are richer in fungal genera, whereas samples from *A. flavicollis* are less diverse. This corresponds with findings of Kataržytė and Kutorga (2011) who also found higher species diversity in samples from *M. glareolus* compared to *A. flavicollis.*

The bimodality of Shanon's diversity index distributions presented in this study indicates that there are apparently two sub-distributions combined together, although there is no method to separate the two fractions from one another. The two distributions suggest that there are two foraging strategies among individuals of both species. The first strategy corresponds to feeding on fungi accidentally and eating only

one of the most common species while foraging on different types of food. The second strategy is connected to active searching for fruit bodies and a diet diverse in fungal genera.

This corresponds to a number of studies suggesting that the key aspect of mammalian mycophagy is the diversity of the diet. Animals thought to be at least occasionally mycophagous feed on a variety of fungal genera. They also do not feed only on fungi, but rather have a balanced, mixed diet with plant and animal material. This way animals maintain their body mass; and their nitrogen intake from the fungi is sufficient enough to sustain them (Maser et al. 1978a; Cork, Kenagy 1989; Johnson 1994, 1996; Claridge et al. 1999; Trappe, Claridge 2005; D'Alva et al. 2007). Consuming a variety of fungal species allows the animals to be more independent of fruiting times of different species, as they do not rely on one species, but can always find a currently fruiting fungus. This makes hypogeous fungi a more reliable food source and lessens the searching effort for animals (Maser, Maser 1988a; Schickmann et al. 2014).

The strategy of accidental mycophagy is dominant in both studied populations, whereas the strategy of active searching is observable during summer. The most statistically significant observations came from the *A. flavicollis* population in Spała, but this may be due to the largest proportion of these samples in the overall number. Nevertheless, a general trend is observed that animals feed on hypogeous fungi most frequently and deliberately in summer.

Foraging or not on any kind of food is always an outcome of balance between nutrition and energy costs. When nutrition of a given food item is not sufficient to cover the energy cost of foraging and the animal's energy demand, the item is not a good food source. For rodents, the nutrition of hypogeous fungi lie in their seasonal availability and relative easiness in finding. Fungi tend to occur abundantly in some periods of the year, but some species are accessible throughout the year. Additionally hypogeous fungi produce sporocarps in large number in certain places called "nests" and emit specific and intensive odours attractive to mammals, additionally luring the animals to these nests (Vogt et al. 1981; Cork, Kenagy 1989; Johnson 1994, 1996; North et al. 1997; Gomez et al. 2003). Therefore fungal sporocarps may be nutritionally important as seasonal food, especially in seasons when more high quality food like seeds and nuts are unavailable (Drożdż 1966; Holišová 1971; Hansson, Larsson 1978; Ure, Maser 1982; Hanssen 1985a; Cork, Kenagy 1989; North et al. 1997).

From a nutritional point of view fungi contain high concentrations of nitrogen, minerals (eg. iron, phosphorus, magnesium), organic compounds like amino-acids, steroids, amines (Claridge 2002), microelements, lipids, vitamins and are also a source of water (Fogel, Trappe 1978; Vogt et al. 1981; Trappe et al. 2009). They contain hormone-like organic compounds, and may be the only source of these components in the environment (Cork, Kenagy 1989). The concentration of minerals and nitrogen in fungi is higher than in leaves and fruits, but their availability to animals is generally low (Vogt et al. 1981; Cork, Kenagy 1989; Claridge et al. 1999; Trappe, Claridge 2005; D'Alva et al. 2007). Studies on fungal nutrition report that about 50-80% of nitrogen in fungi is in non-digestible spores and from the remaining amount half is in non-protein form and carbohydrates constructing the cell wall, which are also not digestible to mammals. During digestion only about 50% of energy from the sporocarp is assimilated. This however can be enhanced for some mammals due to digestive specialization (Cork, Kenagy 1989; Claridge et al. 1999). Claridge et al. (1999) researched nutritional value of a hypogeous species *Rhizopogon viricolor* in a feeding experiment and compared the digestion of fungal material for the Californian vole *Myodes californicus* and the northern flying squirrel, *G. sabrinus*, both reported as preferential mycophages. Their results have shown that, although the animals favoured fungi in their diet, they lost weight when fed only this type of food. They also indicated that voles are adapted to digesting fungal matter and reducing nitrogen loss in faeces more efficiently than the flying squirrels. Another feeding experiment by D'Alva et al. (2007) conducted on mice from genus *Peromyscus* confirmed that animals loose body mass when fed only fungi. These feeding experiments suggest that at least for rodents fungi, both hypo- and epigeous, are of moderate nutritional value. It is because these animals have a hindgut fermentation and are not adapted to digest non-protein structures of fungal cell walls and extract nitrogen from them. On the contrary, marsupials such as rat kangaroos and wallabies have an expended forestomach and have a foregut fermentation, which prolongs their digestive process and allows them to take down cell wall structures, making them suited for a fungal dominated diet (Claridge, Cork 1994, Claridge et al. 1999; D'Alva et al. 2007; Danks 2012).

In comparison with studies in forests of America and Australia, European mammals seem to practice mycophagy to a mild degree. In fact, it was already suggested that there are no obligatory fungivore species in Central Europe (Schickmann et al. 2014). This may be due to ecosystem differences and differences in species

occupying specific niches. In North America ground squirrels occupy the ecological niche which in Europe is typically occupied by voles and mice. Ground squirrels are better adapted to mycophagy than European rodents (Claridge et al. 1999). Their adaptations may not only be anatomical, but also lie in foraging habits (Claridge, Cork 1994). Squirrels are known to store food for a latter time, and flying squirrels were even observed drying fungal sporocarps in tree brunches. Analysis of the sporocarps suggest that fungi may increase their digestibility over time, due to autolysis of cell walls (Trappe et al. 2009). Red-backed voles in North America (*M. californicus* and *M. gapperi*) also cache hypogeous fungi (Ure, Maser 1982) and it is well established that *M. californicus* is a preferential mycophage (Maser, Maser 1988b). *M. glareolus* on the other hand does not store food and if it does, it does not use it fully (Sokolov 1981). It may be that European animals have different foraging habits that exclude them as obligatory mycophages.

Australia is yet another ecosystem with different habitats and different organisms in different niches (Claridge, Cork 1994). It is a continent with the typically seasonal rainfall which can cause seasonal soil deficiencies in nutrients for plant growth. Animals living there have different food strategies than those in the North Hemisphere to utilize seasonal availability of food sources (Taylor 1992; Claridge 2002).

6.4. Hypogeous and epigeous domination in animal diet

In studies referenced in this dissertation, hypogeous fungi dominated over those epigeous in faecal samples (Maser et al. 1978a; Ure, Maser 1982; Ovasaka, Herman 1986; Rhodes 1986; North et al. 1997; Bertolino et al. 2004; Kataržytė, Kutorga 2011; Schickmann et al. 2012). This is explained by a theory that hypogeous fungi are a more stable food source than the epigeous fungi, as they last longer in dry conditions, protected by the humus layer, whereas epigeous fungi are abundant only in autumn and after an onset of rain (Maser et al. 1978b; North et al. 1997; Izzo et al. 2005). In the presented study however, the majority of samples did not contain hypogeous spores, but did contain epigeous spores in various degrees. When looking at the reduced values of the number of samples in classes (showing only presence and absence of hypogeous spores), one can see that the highest number of samples containing hypogeous spores is in the first and second epigeous class. In the

zero epigeous class there are hardly any hypogeous spores, and in third epigeous class there is less than in the previous two classes. This corresponds to the fact that mycophagous mammals indeed feed on a variety of species in a mild amount. Whether or not these are hypo- or epigeous species dependents on the type of environment (Ure, Maser 1982).

In the analysed samples from the first hypogeous class the dominant hypogeous genus is *Glomus*, which may suggest that spores were ingested together with plant material, as *Glomus* forms endomycorrhiza mainly with herb roots. In samples with higher general number of spores, indexes of dominance, frequency and ecological importance shift from the endomycorrhizal *Glomus* to ectomycorrhizal *Rhizopogon* and *Hymenogaster*. On this basis one can assume those genera make the core of hypogeous fungi in the study area, however, it must be borne in mind, that results of faecal analysis do not point to biomass production in the environment, but rather the proportional representation of consumed taxa (Remick 2015).

Basidiomycetes are dominant among hypogeous fungi found in animal samples, accounting for 61% of spores found (Maser et al. 1978a). In the study of red squirrels, Bertolino et al. (2004) and Jacobs and Luoma (2008) also found that the most frequent genus in animal samples was *Rhizopogon*. Izzo et al. (2005) found *Rhizopogon, Melanogaster*, and *Gautieria* being the most abundant in their samples. Orrock and Pagels (2002) in their study noted that *Melanogaster* was the most frequent, *Elaphomyces* was close second and *Hymenogaster* was least frequent.

The frequency of some basidiomycetes is linked to the study area. In their work on sporocarp production in forests, Colgan et al. (1999) discovered that light thinning and forest management promote formation of *Melanogaster* and *Rhizopogon* sporocarps in a local scale, but causes the decline in other species like *Gauteria*. Izzo et al. (2005) also stated that the genus *Rhizopogon* occurs in disturbed environments and be absent from mature stands. Both of the study plots in Spała and Konewka are in managed forests with heavy anthropogenic influence, which creates a microclimate suitable for development of specific species like *Rhizopogon.*

North et al. (1997) suggested that consumption of various species change due to their abundance, but also depends on the diversity of overall sporocarps available in the environment and on the availability of alternative food items. The Author of this study would add that spore presence in faecal samples is derived not only from the presence of fruit bodies in the habitat, but also from aspects such as their attractiveness

for the animals and the structure of fruit bodies. North et al. (1997) postulated that high abundance of *Elaphomyces granulatus*, which is slow to decay and linger in the soil makes it an important food reservoir for mycophages. This however is not supported by this study, where *Elaphomyces* is not frequent in samples. Kataržytė and Kutorga (2011) also found that although *Elaphomyces* is abundant in the environment, it is not abundant in samples form animals, and found only in spring samples, when other species were scarce. Johnson (1994) suggests that the reason why animals – in his study the Australian rat-kangaroo *Bettongia gaimardi* – do not feed often on *Elaphomyces* is because of its faint aroma. It also may be that the low frequency of *Elaphomyces* is due to dispersal strategy of the fungus, as it is partly wind-dispersed. When mycophages open the sporocarp, the powdery mass of spores is freed into the air, enabling additional dispersion when animals feed on the peridium (Schickmann et al. 2014). This could be linked with the structure of the *Elaphomyces* sporocarp, which may be not attractive to animals. In such a case, the animal opens the sporocarp and eats some part of the peridium, but leaves the powdery spore mass, allowing dispersion by wind (Trappe et al. 2009). Even though animals mostly eat the peridium, samples taken from them still contain some amounts of mature spores (Trappe, Maser 1976).

Although in the analysed samples *Glomus* was one of the more frequent genera, the second endomycorrhizal genus – *Endogone* was among the least frequent. This may be due to the fact, that *Endogonaceae* produce a fainter odour and are smaller than other hypogeous fungi (Ure, Maser 1982), but also to changes in nomenclature, where some *Endogone* fungi were included to *Glomus* (Meyer et al. 2015)

6.5. Morphometric characteristics and ornamentation

The observation of seasonal morphometric diversity of spores turned out to be inconclusive. The first condition for ANOVA I testing of morphometric diversity was for spores to occur in all three seasons and the second was the sufficient number of measured spores. The only statistically significant result of ANOVA I testing was for the genus *Rhizopogon.* Spores of this genus increased in size from spring and through summer and autumn. This may suggest that *Rhizopogon* is available to animals throughout the year and in great numbers, unlike some genera like *Pachyphloeus* and *Genea*, which were in samples only in one, given season. This however needs to be confirmed in further studies on mycophagy combined with fenological studies on

hypogeous fungi. Studying faecal samples gives scientists more information about the taxa found in the given terrain, but in the process some taxonomical information is lost. Most spores found in animals can be identified to genus level, but identification to species may be problematic (Remick 2015). One of the most characteristic features of hypogeous spores is their ornamentation. The patterns of the ornamentation are specific for each genus, which is what makes hypogeous fungi identifiable under the microscope. However it is sometimes insufficient to identify spores to species level, as their colour, size and ornamentation change with age. This makes identification difficult in case of immature spores. The ornamentation can also partly dissolve after passage through the animal gut. This is a source of speculation, that the ornamentation serves as a protective barrier against spore digestion (Claridge et al. 1992; Trappe, Claridge 2005; Trappe et al. 2009). In this study the Author's observation on the matter is that under the microscope spores were rarely found in clear suspension, but usually in the sediment. Under the microscope, spores were found in fields where faecal material was gathered. The Author therefore presumes that ornamentation of the spores enables their enclosing in faecal material. After passing through the animal gut, the gathered material in spaces between the ornamentation may serve as a nutrient reservoir for the spores during further development in the soil (Li et al. 1986).

6.6. Final remarks

Hypogeous fungi are a very interesting group to study by mycologists. They are frequently found in mycorrhizal samples and samples from animals, but less frequently as sporocarps. Often more hypogeous taxa are found in animal samples than was reported found in the environment by researchers (Johnson 1994; Pyare, Longland 2001; Izzo et al. 2005; Kataržytė, Kutorga 2011; Remick 2015).

As a lot of mycorrhizal fungi are consumed by animals, making mycorrhiza and mycophagy inseparable phenomena, influencing the structure, functioning and stability of the forest ecosystem (Johnson 1996). It is highly possible that mycophagy is a network-phenomenon, like pollination and frugivory. This indicates a high specialization of the organisms involved – fungi produce aromas attractive to mycophages and animals develop metabolic mechanisms suitable to digest fungal material. The phenomenon itself and its role in the forest ecosystem is still not fully understood (Maser et al. 1985; Trappe at al. 2009; Schickmann et al. 2014). As it is with

all complex networks, a disruption in any of the levels constructing this system will inevitably impact others and by this influence other elements of the forest ecosystem such a predators (Maser et al. 1985; North et al. 1997; Trappe et al. 2009). In this field of study, there are still aspects of animal-fungus-tree relationship that science has yet to uncover. It is still inconclusive, how these relations influence the resilience and productivity of the whole ecosystem. Interactions between mammals and fungi may have an important role in retaining forest health, and the regeneration of forest stands after clear cuts, where there is a need for reinoculation with spores (Maser et al. 1985; Pyare, Longland 2001; Trappe, Claridge 2005; Jacobs, Luoma 2008; Trappe et al. 2009; Schickmann et al. 2014). Legal protection of hypogeous fungi has recently shifted from species protection to habitat protection, so to protect fungi as well as their mycophages, mycorrhizal hosts, and ceased wood, which also benefits, both fungi and animals (Grzywacz 2003; Trappe et al. 2009; Ławrynowicz 2014-2015)

A question remains about the factors that influence the germination of spores after passing through the animal digestive tract. In particular, how gut retention, body temperature, anatomical differences and animal physiology influence the development of the mycelium itself and the formation of mycorrhizae. These are particularly difficult questions, as testing the hypothesis for them is problematic in the natural environment (Claridge et al. 1992; Colgan, Claridge 2002). There also remain questions of ecological nature, like how animal activity influences trees and fungi, both negative (eating the roots) and positive (aeration and turning of the soil).

The forest ecosystem in its whole is composed of living organisms and is regulated by interactions between them. Animals depend on plants for food, shelter and breeding places. In turn, the growth of plants is conditioned by mycorrhizal fungi. Therefore, the forest should be considered as a system with overlapping trophic and symbiotic interactions where every component benefits from the ecosystem and has an input on its prolonged functioning (Maser et al. 1985; Maser, Maser 1988a). Fungi play an important binding role in this system, through mycorrhiza linking hyphae with plants and through mycophagy connecting sporocarps to animals (Maser, Maser 1988a). Only an integrated research, combining knowledge of various natural sciences (mycology, zoology, botany, ecology) can provide methods for conducting studies for better understanding, protection, restoration and management of the forest resources (McCreary 2004; Izzo et al. 2005; Nathan 2006; Trappe et al. 2009; Ławrynowicz 2014-2015).

7. CONCLUSIONS

The recorded seasonal differentiation in samples shows that hypogeous fungi may be a significant element in the diet of forest rodents. In particular, the Author concludes:

- In spring and autumn, *M. glareolus* and *A. flavicollis* eat the most common hypogeous fungi, and in summer season both species consume significantly more fungi from a multitude of genera. The amount of found spores follows the patterns of high temperature and mild rainfall.
- The mean number of spores found in samples was higher in Spała than in Konewka. This may be due to more favourable microhabitat conditions in Spała, especially for hypogeous ascomycetes.
- Significantly more spores were found in samples from traps which were closer to the road. This corresponds to the fact that close proximity to trampled pathways limits plant vegetation and favours formation of sporocarps.
- *M. glareolus* is more mycophagous than *A. flavicollis*, as samples from voles were richer in fungal genera the those from mice.
- Young specimens of both species eat more hypogeous fungi than adults. The young are more likely to forage closer to the ground and due to their mobility, they play an important role in spore dispersion.
- Females of *A. flavicollis* eat more hypogeous fungi than males. Females are more sedentary than males and forage in places suitable for fruiting of hypogeous fungi.
- Spores of genus *Rhizopogon* were observed to increase in size from spring through summer and autumn. This suggests that this genus is available to animals throughout the year unlike some genera which were found in samples only in one given season.

8. SUMMARY

Hypogeous fungi are a ecological group which congregates various fungal genera from Ascomycota, hypogeous 'gasteromycetes' from Basidiomycota and a few taxa from Glomeromycota. Though taxonomically distant, hypogeous fungi show features of convergent evolution in habitat adaptations, because they occupy a specific ecological niche. They produce underground, closed macroscopic fruit bodies, and are important in the forest ecosystem due to their role as ectomycorrhizal partners for plants, especially forest trees. As their sporocarps remain close, the fungi rely mostly on animals as vectors of dispersion. In case of hypogeous ascomycetes, the asci have no opening mechanisms and remain closed until natural decay or digestion by animals.

Hypogeous fungi produce characteristic odours, detectable by animals which feed on them. Most mammals are opportunistic or accidental mycophages which means they feed on fungi when this type of food is abundant in the environment or while foraging for other food source. Examples of mycophages can be found in the mouse family Muridae and the vole family Arvicolinae.

The aim of this study is to examine the significance of hypogeous fungi in diet of rodents in the forest ecosystem of Central Poland. The study will verify the hypothesis that hypogeous fungi are an important component of rodent diet and that mycophagy plays a significant role in the forest ecosystem. For this purpose, the Author examined the occurrence of spores in faecal samples from two species of rodents: bank vole *Myodes glareolus* and yellow-necked mouse *Apodemus flavicollis*. Both species are widely spread in the Palaearctic, abundant in forest ecosystems and are reported as preferential or opportunistic mycophages. In particular the following issues were of a special concern (1) the diversity of fungal genera in faecal samples; (2) difference in spore occurrence in samples obtained in three seasons: spring, summer and autumn; (3) differences in spore occurrence in relation to study area, animal species, sexes and age.

This is the first study of this kind conducted in central Poland and is based on original field research and microscope analysis of samples gathered in the field.

The study was carried out by live trapping animals. The study was conducted in the Spała (51°31'37" N 20°08'42" E) and Konewka (51°04'08" N 20°09'26" E) nature reserves, located in Pilica Forest, in Łódzkie Voivodship in central Poland, between July 2013 and May 2015. Once an animal was caught, its species was

determined. After the capture, animals were weighed and their sex and age group (juvenile or adult) were determined for later comparisons. The animals were marked with a red dot on the abdomen and released. Faecal samples were then taken from the live traps and placed in a 1,5 ml Eppendorf tube with 1 ml of 90% ethanol for preservation. Samples were examined using NIKON E200 light microscope under x600 magnification. Spores of hypogeous fungi which were found in samples were determined to genus level. Twelve hypogeous genera were present in the samples: *Elaphomyces, Hydnotrya, Pachyphloeus, Genea, Tuber, Hymenogaster, Melanogaster, Rhizopogon, Scleroderma, Gautieria, Glomus* and *Endogone*.

Statistical analysis of seasonal differences in animal diet as well as differences between animal's species, age and sex has shown that in spring and autumn, *M. glareolus* and *A. flavicollis* eat the most common genera of hypogeous fungi, and in summer season both species consume significantly more fungi from a multitude of genera. The amount of found spores follows the patterns of high temperature and mild rainfall. The mean number of spores found in samples was higher in Spała than in Konewka. This may be due to more favourable microhabitat conditions in Spała, especially for hypogeous ascomycetes. Significantly more spores were found in samples from traps which were closer to the road. This corresponds to the fact that close proximity to trampled pathways limits plant vegetation and favours formation of sporocarps. *M. glareolus* is more mycophagous than *A. flavicollis*, as samples from voles were richer in fungal genera the those from mice. Young specimens of both species eat more hypogeous fungi than adults. The young are more likely to forage closer to the ground and due to their mobility, they play an important role in spore dispersion. Also females of *A. flavicollis* eat more hypogeous fungi than males. Females are more sedentary than males and forage in places suitable for fruiting of hypogeous fungi.

Spores of genus Rhizopogon were observed to increase in size from spring through summer and autumn. This suggests that this genus is available to animals throughout the year unlike some genera which were found in samples only in one given season. The Author also presumes that the ornamentation on the surface of the spores may serve as a reservoir for nutritional material from the faeces.

9. STRESZCZENIE

Grzyby podziemne to grupa ekologiczna składająca się z różnych gatunków grzybów spośród Ascomycota, Basidiomycota oraz kilku rodzajów z Glomeromycota. Choć taksonomicznie odległe, należące do tej grupy grzyby wykazują zbieżne cechy ewolucyjne w przystosowaniu do środowiska, co wynika z zajmowania przez nie specyficznej niszy ekologicznej. Grzyby te wytwarzają podziemne, zamknięte, makroskopowe owocniki i są istotne dla ekosystemu leśnego ze względu na swoją rolę jako partnerzy ektomykoryzowi dla roślin, głownie drzew lasotwórczych. Ze względu na fakt, że ich owocniki pozostają zamknięte, w zakresie rozprzestrzeniania się grzyby te głównie wykorzystują zwierzęta. W przypadku Ascomycota, worki nie posiadają mechanizmów otwierających, pozostając zamknięte aż do samoistnego rozpadu lub strawienia przez zwierzę.

Grzyby podziemne wydzielają charakterystyczny zapach, wyczuwalny przez zwierzęta, które się nimi żywią. Większość ssaków zalicza się do przypadkowych lub oportunistycznych mykofagów, co oznacza, że żywią się grzybami wtedy, gdy te obficie występują w środowisku lub też gdy znajdują je szukając innego pożywienia. Przykłady mykofagów można znaleźć wśród myszy z rodziny Muridae i norników z rodziny Arvivolinae.

Celem badań przedstawionych w rozprawie jest ocena znaczenia grzybów podziemnych w diecie gryzoni w ekosystemach leśnych w Polsce Środkowej, w szczególności potwierdzenie hipotezy o znacznym udziale grzybów podziemnych w diecie gryzoni, a co za tym idzie ważnej roli mykofagii w ekosystemie leśnym. W tym celu Autorka zbadała obecność zarodników w próbkach odchodów zebranych od dwóch gatunków gryzoni: nornicy rudej *Myodes glareolus* oraz myszarki leśnej *Apodemus flavicollis*. Oba gatunki badanych zwierząt są szeroko rozprzestrzenione w Palearktyce i występują powszechnie w ekosystemach leśnych. Uważa się je również za preferencyjnych lub oportunistycznych mykofagów. W szczególności zbadano następujące zagadnienia: (1) różnorodność taksonomiczne grzybów w próbkach; (2) różnice w pojawianiu się zarodników w próbach z trzech pór roku: wiosny, lata i jesieni; (3) różnice w obecności zarodników w zależności od terenu badań, gatunku zwierzęcia, jego płci oraz wieku.

Są to pierwsze badania tego rodzaju przeprowadzone w Polsce Środkowej, oparte na oryginalnych badaniach terenowych i analizie mikroskopowej próbek pobranych od zwierząt.

Badanie przeprowadzono metodą chwytania zwierząt w pułapki żywołowne w rezerwatach Spała (51°31'37" N, 20°08'42" E) i Konewka (51°04'08" N, 20°09'26" E), w Puszczy Pilickiej (województwo łódzkie) między czerwcem 2013 a majem 2015 r. Złapane zwierza po wyjęciu z pułapek, oznaczano do gatunku oraz ustalono płeć, wagę i wiek dla późniejszych porównań. Zwierzęta oznaczano czerwoną kropką na stronie brzusznej i wypuszczano. Próbki odchodów były pobierane z pułapek, umieszczone w próbówkach (Eppendorf, 1,5 ml) i utrwalone w 1 ml 90% etanolu. Próbki były analizowane przy użyciu mikroskopu świetlnego NIKON E200 (x600). Znalezione w próbkach zarodniki grzybów podziemnych zostały oznaczone do rodzaju, a gdzie było to możliwe, do gatunku. W próbkach zidentyfikowano dwanaście rodzajów grzybów podziemnych: *Elaphomyces, Hydnotrya, Pachyphloeus, Genea, Tuber, Hymenogaster, Melanogaster, Rhizopogon, Scleroderma, Gautieria, Glomus* oraz *Endogone*.

Analiza statystyczna zróżnicowania sezonowego w diecie gryzoni, jak również porównanie składu ilościowego i jakościowego prób między gatunkami zwierząt, grupami wiekowymi i płciami wykazały, że grzyby podziemne są atrakcyjnym pożywieniem dla zwierząt w czasie letnim. Wiosną i jesienią *M. glareolus* i *A. flavicollis* żywią się pojedynczymi i łatwo dostępnymi rodzajami grzybów, natomiast w lecie zwierzęta konsumują istotnie więcej grzybów z wielu rodzajów. Ilość zarodników znalezionych w próbach korespondowała z występowaniem wysokich temperatur i umiarkowanych opadów. Średnia liczba zarodników była wyższa w próbach zebranych w Spale. Może to wskazywać, że w Spale występują lepsze warunki dla rozwoju owocników niż w Konewce. Ma to szczególne znaczenie dla podziemnych workowców, dla których analiza statystyczna wykazała istotną zależność od terenu badań. Istotnie więcej zarodników znaleziono w próbach pobranych z pułapek ustawionych bliżej drogi. Przemawia to za stwierdzeniem, że owocniki grzybów podziemnych pojawiają się częściej przy wydeptywanych ścieżkach, gdzie roślinność jest przerzedzona i są lepsze warunki do rozwoju grzybów.

Nornice częściej niż muszarki wybierają grzyby jako źródło pokarmu, jako że w próbach od nornic odnotowano większe zróżnicowanie taksonomiczne grzybów, niż w próbach od myszarki. Młode osobniki jedzą więcej grzybów podziemnych niż

osobniki dorosłe. Młode zwierzęta wyszukują pokarm bliżej powierzchni gruntu, a ze względu na swoją mobilność, pełnią w ten sposób istotną rolę w rozprzestrzeniamu zarodników. Również samice myszarki leśnej są bardziej mykofagiczne niż samce. Są one bardziej stacjonarne niż samce i żerują w miejscach odpowiednich dla owocników grzybów podziemnych.

Analiza morfometryczna zarodników z rodzaju *Rhizopogon* wykazała iż zarodniki te zwiększały swoje rozmiary w ciągu roku. Sugeruje to, iż grzyby z tego rodzaju są dostępne dla zwierząt przez cały rok, w odróżnieniu od niektórych rodzajów, które były znajdowane tylko w czasie określonej pory roku. Ponadto Autorka zauważyła, że skulptura na powierzchni zarodników może magazynować substancje odżywcze znajdujące się w odchodach zwierząt.

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10.2. Legislation:

1. Dz. Urz. Woj. Piotrkowskiego 1995.15.113. Rozporządzenie Nr 4/95 Wojewody Piotrkowskiego z dnia 5 października 1995 r. w sprawie utworzenia Spalskiego Parku Krajobrazowego
2. Dz. Urz. Woj. Łódzkiego 2001.206.2976. Obwieszczenia Nr 2/2001 Wojewody Łódzkiego z dnia 2 października 2001 r. w sprawie ogłoszenia wykazu

rezerwatów przyrody na terenie województwa łódzkiego utworzonych do dnia 31 grudnia 1998 r.

3. Dz. Urz. Woj. Łódzkiego 2010.194.1566 Zarządzenie Nr 48/2010 Regionalnego Dyrektora Ochrony Środowiska w Łodzi z dnia 17 czerwca 2010 r. w sprawie rezerwatu przyrody "Konewka"
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6. M. P. 1978.33.126 Zarządzenie Minister Leśnictwa i Przemysłu Drzewnego z dnia 11 października 1978 r. w sprawie uznania za rezerwaty przyrody

10.3. World Wide Web Resources:

1. Czachorowski S. 2006 Opisywanie biocenozy – zoocenologia, Skrypt elektorniczny dla magistrantów (wersja 2 uzupełniona i poprawiona). Olsztyn: http://www.uwm.edu.pl/czachor/publik/pdf-inne/zoocenozy.pdf (8.02.2017)
2. Catalogue of Life: http://www.catalogueoflife.org/col/browse/tree?474e99d189f24428e0449c5d426f2e0c (8.05.2017)
3. Geoserwis GDOŚ: http://geoserwis.gdos.gov.pl/mapy/
4. Index Fungorum: http://www.indexfungorum.org/names/Names.asp (8.05.2017)
5. Tutiempo: https://en.tutiempo.net/climate/ws-124690.html (8.05.2017)

Appendix 1.
Tables for factor analysis.

In presented Tables values marked in red are statistically significant.

Table 1. Factor analysis for weather conditions

Weather conditions	PCA_1	PCA_2
Average temperature	0,968	0,123
Maximum temperature	9,65	0,108
Minimum temperature	0,838	0,458
Humidity	-0,927	0,138
Rainfall	-0,258	0,764
Average wind speed	0,341	0,872
Maximum wind speed	0,363	0,845
Fog	-0720	-0,367
Expo. Var	4,262	2,451
Prp. Totl	0,533	0,306

Table 2. All possible statistical models for weather conditions (PCA1, PCA2), study plot and rodent species, using the Akaike Information Criterion

	Var. 1	Var. 2	Var. 3	Var 4.	dr	AIC	L. Ratio χ^2
1	PCA_1	PCA_2	Study plot	Rodent species	4	2543,8	994,15
2	PCA_1	PCA_2	Rodent species		3	2632,7	903,30
3	PCA_1	Rodent species			2	2654,9	879,11
4	PCA_1	Study plot	Rodent species		3	2668,9	867,10
5	PCA_1	PCA_2			22	2717,3	816,65
6	PCA_1	Study plot			2	2754,4	779,58
7	PCA_1				1	2756,0	775,95
8	PCA_1	PCA_2	Study plot		3	2789,0	746,94
9	Study plot	Rodent species			2	3146,5	387,52
10	PCA_2	Study plot	Rodent species		3	3146,7	389,32
11	PCA_2	Rodent species			2	3269,5	264,47
12	Rodent species				1	3281,5	250,43
13	PCA_2	Study plot			2	3325,2	208,75
14	Study plot				1	3335,0	197,01
15	PCA_2				1	3460,3	71,67

Table 3. Statistics for chosen models from Table 2

Effect	Effect Level	Evaluation	Std. Er.	Walda Stat.	Upper GU 95,0%	Lower GU 95,0%	p
Intercept		5,20034	0,171387	920,6806	4,86443	5,53626	< 0,0001
PCA_1		3,70594	0,128311	834,2005	3,45446	3,95743	< 0,0001
PCA_2		-0,47936	0,047514	101,7817	-0,57248	-0,38623	< 0,0001
Study plot	1	1,49856	0,056332	707,6934	1,38816	1,60897	< 0,0001
Rodent species	1	-1,82462	0,117337	241,8062	-2,05458	-1,59463	< 0,0001
Scale		1,00000	0,000000		1,00000	1,00000	

Table 4. Analysis of diversity of ascomycetous spores in relation to weather conditions (component 1-2), study plot and rodent species

Effect	df	Wald Stat.	p
Intercept	1	92,15	<0,0001
PCA_1	1	58,17	<0,0001
PCA_2	1	53,13	<0,0001
Study plot	1	11,50	0,001
Rodent species	1	2,51	0,113

Table 5. Analysis of diversity of basidiomycrtous spores in relation to weather conditions (component 1-2), study plot and rodent species

Effect	df	Wald Stat.	p
Intercept	1	812,92	<0,0001
PCA_1	1	723,54	<0,0001
PCA_2	1	431,04	<0,0001
Study plot	1	0,07	0,936

Table 6. Analysis of diversity of glomeromycetous spores in relation to weather conditions (component 1-2), study plot and rodent species

Effect	df	Wald Stat.	p
Intercept	1	105,00	<0,0001
PCA_1	1	15,36	<0,0001
PCA_2	1		

Table 7. All possible statistical models for rodent species and sex, using the Akaike Information Criterion

	Var. 1	Var. 2	Var. 3	df	AIC	L. Ratio χ^2
1	Species	Sex	Species*sex	3	3052,509197	128,663173
2	Species	Species*sex		2	3055,809821	123,362549
3	Species	Sex		2	3055,950506	123,221864
4	Species			1	3094,745003	82,427367
5	Sex	Species*sex		2	3114,325474	64,846896
6	Species*sex			1	3128,043355	49,129015
7	Sex			1	3137,762687	39,409683

Table 8. All possible statistical models for rodent species and age, using the Akaike Information Criterion

	Var. 1	Var. 2	Var. 3	df	AIC	L. Ratio χ^2
1	Species	Age	Species*age	3	3403,675839	263,443703
2	Species	Age		2	3407,717596	257,401946
3	Species	Species*age		2	3434,971028	230,148513
4	Species			1	3435,089222	228,030319
5	Age	Species*age		2	3529,914375	135,205166
6	Age			1	3560,376147	102,743394
7	Species*age			1	3623,938522	39,181019

Appendix 2.
Soil analysis

Table 1. Soil analysis in the study area: pH in H_2O, pH in KCl, presentage of organic compunds (N, C and organic matter) and K_2O, P_2O_5, CaO, K, Na, Ca and Mg in mg per 100 g of soil. Data gather from 10 plots in each study plot (Spała S 1-10 and Konweka K 1-10) (Ławrynowicz, Mleczko unpublished data).

Lp	pH in H_2O	pH in KCl	org. N	org. C	org. mat	K_2O	P_2O_5	CaO	K	Na	Ca	Mg
S 1	5,64	4,22	0,380	3,823	6,591	4,0	10	16,8	1,4	2,3	12	1,0
S 2	5,03	3,96	0,290	5,639	9,722	3,6	13,2	22,4	1,4	2,4	16	1,6
S 3	4,60	3,66	0,287	6,155	10,396	5,0	7,6	30,8	2,0	0,9	22	2,4
S 4	4,53	3,55	0,391	7,325	12,628	9,6	7,0	37,8	4,8	2,3	27	2,2
S 5	4,57	3,60	0,419	7,550	12,300	5,8	5,4	28,0	2,0	2,8	20	1,6
S 6	3,96	3,68	0,285	5,081	8,760	8,6	9,4	25,2	3,0	1,1	18	3,0
S 7	4,68	4,02	0,167	2,950	5,172	5,8	9,4	25,2	2,0	1,1	18	2,4
S 8	5,14	4,13	0,304	5,493	9,470	5,0	4,4	25,2	2,6	1,3	18	0,6
S 9	5,01	4,03	0,674	10,85	18,705	12,2	10,4	75,6	8,6	1,6	54	3,0
S 10	4,37	3,36	0,299	5,041	8,708	5,8	3,2	44,8	2,6	0,9	32	1,0
K 1	5,62	4,74	0,133	2,440	4,206	11,4	8,0	98	7,4	2,1	70	5,0
K 2	5,61	4,55	0,148	2,330	4,017	9,8	10,6	50,4	6,2	2,8	36	3,0
K 3	5,12	3,90	0,360	7,000	12,068	41,5	21,6	103,6	30,6	2,3	74	8,0
K 4	5,69	4,62	0,146	2,653	4,574	10,4	14,0	47,6	7,0	2,4	34	3,0
K 5	5,84	4,55	0,141	2,226	3,839	12,6	17,8	36,4	9,2	2,8	26	4,0
K 6	5,91	4,67	0,119	1,962	3,382	8,6	12,6	42,0	5,8	2,3	30	3,6
K 7	5,25	4,31	0,294	5,259	9,067	10,2	21,6	162,4	7,0	3,8	116	5,4
K 8	5,32	4,34	0,155	2,371	4,088	10,4	12,6	61,6	5,4	1,6	44	0,0
K 9	4,60	3,67	0,195	3,021	5,592	6,2	13,2	28,0	2,6	0,9	20	0,0
K 10	5,03	4,06	0,366	6,959	11,997	17,6	20,4	109,2	12,2	3,8	78	0,0

Appendix 3.
Morphometrical analysis of spores found in faecal samples

Table 1. Descriptive statistics for length and width of *Elaphomyces* spores, with N (number of spores measured), Std. D. (standard deviation) and Std. Er. (standard error).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	60	19,53	13,33	23,33	2,23	-0,26	0,31	0,26	0,61
Width	60	18,99	13,33	23,33	2,33	-0,10	0,31	-0,42	0,61
CI	60	0,97	0,83	1,08	0,05	-0,85	0,31	0,42	0,61

Table 2. Descriptive statistics for length and width of *Elaphomyces* spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	36	20,64	18,33	23,33	1,56	0,49	0,393	-0,64	0,79
Width	36	19,99	16,25	23,33	1,97	-0,044	0,393	-0,63	0,77
CI	36	0,96791	0,83	1,083	0,05	-0,60	0,393	0,29	0,77

Table 3. Descriptive statistics for length and width of *Elaphomyces* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	24	17,88	13,33	23,33	2,070	0,22	0,472	1,36	0,92
Width	24	17,50	13,33	21,67	2,03	0,00	0,472	-0,34	0,92
CI	24	0,98	0,90	1,00	0,037	-1,39	0,472	0,089	0,92

Table 4. Descriptive statistics for length and width of *Hydnotrya* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	42	33,97	18,33	46,67	6,21	-0,36	0,37	0,02	0,72
Width	42	30,16	13,33	38,33	5,52	-0,67	0,37	0,51	0,72
CI	42	0,89	0,70	1,00	0,08	-0,49	0,37	-0,35	0,72

Table 5. Descriptive statistics for length and width of *Hydnotry*a spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	4	36,25	31,67	41,67	5,34	0,084	1,014185	-5,52	2,62
Width	4	30,42	25,00	38,33	5,67	1,199	1,014185	1,98	2,62
CI	4	0,84	0,75	0,92	0,082	-0,12	1,014185	-4,44	2,62

Table 6. Descriptive statistics for length and width of *Hydnotrya* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	33	35,05	25,00	46,67	5,13	0,20	0,41	-0,28	0,80
Width	33	31,26	25,00	38,33	4,29	-0,12	0,41	-1,25	0,80
CI	33	0,90	0,70	1,00	0,08	-0,49	0,41	-0,03	0,80

Table 7. Descriptive statistics for length and width of *Hydnotrya* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	5	25,00	18,33	36,67	6,97	1,54	0,91	2,92	2,00
Width	5	22,67	13,33	35,00	7,78	0,94	0,91	2,47	2,00
CI	5	0,90	0,73	1,00	0,11	-1,20	0,91	1,38	2,00

Table 8. Descriptive statistics for length and width of *Genea* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	21	28,81	23,33	35,00	3,81	-0,08	0,50	-0,97	0,97
Width	21	22,30	16,67	31,67	2,91	1,28	0,50	5,08	0,97
CI	21	0,78	0,62	1,00	0,11	0,66	0,50	-0,23	0,97

Table 9. Descriptive statistics for length and width of *Tuber* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	23	25,76	16,67	48,33	6,99	2,13	0,48	5,00	0,94
Width	23	20,21	13,33	40,00	6,04	2,04	0,48	5,10	0,94
CI	23	0,79	0,62	1,00	0,11	0,17	0,48	-1,01	0,94

Table 10. Descriptive statistics for length and width of *Tuber* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	21	23,93	16,67	33,33	3,47	0,65	0,50	2,09	0,97
Width	21	18,64	13,33	25,00	3,05	-0,08	0,50	-0,42	0,97
CI	21	0,78	0,62	1,00	0,11	0,14	0,50	-0,86	0,97

Table 11. Descriptive statistics for length and width of *Tuber* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.
Length	2	45,00	41,67	48,33	4,71
Width	2	36,67	33,33	40,00	4,72
CI	2	0,83	0,69	0,96	0,19

Table 12. Descriptive statistics for length and width of *Hymenogaster* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	168	18,55	10,00	33,33	3,47	0,69	0,19	1,36	0,37
Width	168	10,19	6,67	20,00	2,03	1,35	0,19	3,65	0,37
CI	168	0,56	0,33	0,90	0,10	0,61	0,19	0,89	0,37

Table 13. Descriptive statistics for length and width of *Hymenogaster* spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	48	19,58	13,33	33,33	4,01	1,13	0,34	1,82	0,67
Width	48	10,14	6,67	15,00	1,73	0,24	0,34	0,26	0,67
CI	48	0,53	0,40	0,90	0,09	1,57	0,34	5,53	0,67

Table 14. Descriptive statistics for length and width of *Hymenogaster* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	105	18,01	10,00	25,00	3,19	0,22	0,24	-0,19	0,47
Width	105	10,25	6,67	20,00	2,23	1,54	0,24	3,66	0,47
CI	105	0,58	0,33	0,90	0,10	0,24	0,24	0,29	0,47

Table 15. Descriptive statistics for length and width of *Hymenogaster* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	15	19,00	15,00	23,33	2,87	-0,24	0,58	-1,49	1,12
Width	15	9,94	8,33	11,67	1,36	0,01	0,58	-1,49	1,12
CI	15	0,53	0,42	0,67	0,07	0,42	0,58	0,18	1,12

Table 16. Descriptive statistics for length and width of *Melanogaster* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	64	14,81	10,00	28,33	3,52	2,53	0,30	7,28	0,59
Width	64	8,28	6,67	15,00	1,54	2,40	0,30	7,47	0,59
CI	64	0,57	0,42	0,86	0,08	0,97	0,30	1,86	0,59

Table 17. Descriptive statistics for length and width of *Melanogaster* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	61	14,37	10,00	27,50	2,73	2,72	0,31	11,45	0,60
Width	61	8,13	6,67	15,00	1,32	2,83	0,31	13,00	0,60
CI	61	0,57	0,42	0,86	0,08	0,97	0,31	1,90	0,60

Table 18. Descriptive statistics for length and width of *Melanogaster* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.
Length	2	27,50	26,67	28,33	1,17
Width	2	12,92	12,50	13,33	0,59
CI	2	0,47	0,44	0,50	0,04

Table 19. Descriptive statistics for length and width of *Rhizopogon* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	202	15,57	10,00	20,00	1,72	-0,08	0,17	0,46	0,34
Width	202	6,60	5,00	16,67	1,16	3,60	0,17	28,84	0,34
CI	202	0,43	0,27	1,00	0,07	2,45	0,17	17,10	0,34

Table 20. Descriptive statistics for length and width of *Rhizopogo*n spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	7	15,00	11,67	18,33	2,54	0,39	0,79	-1,12	1,59
Width	7	6,67	5,00	8,333	0,96	-0,01	0,79	3,00	1,59
CI	7	0,45	0,36	0,56	0,07	0,03	0,79	-1,23	1,59

Table 21. Descriptive statistics for length and width of *Rhizopogon* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	191	15,51	10,00	20,00	1,62	-0,28	0,18	0,49	0,35
Width	191	6,56	5,00	16,67	1,14	3,94	0,18	33,18	0,35
CI	191	0,43	0,27	1,00	0,07	2,59	0,18	18,16	0,35

Table 22. Descriptive statistics for length and width of *Rhizopogon* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	4	19,17	16,67	20,00	1,67	-2,00	1,01	4,00	2,62
Width	4	8,33	6,67	10,00	1,36	0,01	1,01	1,50	2,62
CI	4	0,44	0,33	0,50	0,08	-0,85	1,01	-1,31	2,62

Table 23. Descriptive statistics for length and width of *Scleroderma* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	12	15,00	8,33	20,00	3,26	-0,53	0,64	0,32	1,23
Width	12	14,58	8,33	20,00	3,19	-0,23	0,64	0,20	1,23
CI	12	0,97	0,89	1,00	0,05	-1,37	0,64	-0,10	1,23

Table 24. Descriptive statistics for length and width of *Scleroderma* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	11	15,00	8,33	20,00	3,42	-0,51268	0,66	0,017	1,28
Width	11	14,70	8,33	20,00	3,32	-0,34687	0,66	0,08	1,28
CI	11	0,98	0,89	1,00	0,04	-1,98011	0,66	2,45	1,28

Table 25. Descriptive statistics for length and width of *Gautieria* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	30	16,50	11,67	21,67	2,75	0,47	0,43	-0,57	0,83
Width	30	9,94	6,67	15,00	1,92	0,96	0,43	0,59	0,83
CI	30	0,61	0,42	0,75	0,10	-0,34	0,43	-1,04	0,83

Table 26. Descriptive statistics for length and width of *Gautieria* spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	4	15,00	13,33	16,67	1,36	0,00	1,01	1,50	2,62
Width	4	9,375	8,33	10,00	0,80	-0,87	1,01	-1,24	2,62
CI	4	0,63	0,56	0,69	0,06	-0,34	1,01	-3,05	2,62

Table 27. Descriptive statistics for length and width of *Gautieria* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	25	16,60	11,67	21,67	2,83	0,40	0,46	-0,59	0,90
Width	25	10,03	6,67	15,00	2,08	0,81	0,46	0,01	0,90
CI	25	0,61	0,42	0,75	0,11	-0,37	0,46	-1,13	0,90

Table 28. Descriptive statistics for length and width of *Glomus* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	82	61,78	21,67	166,67	28,47	1,04	0,27	1,74	0,53
Width	82	55,48	6,00	143,33	26,68	1,03	0,27	1,16	0,53
CI	82	0,91	0,09	1,43	0,14	-1,96	0,27	17,34	0,53

Table 29. Descriptive statistics for length and width of *Glomus* spores found in spring (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	17	34,22	21,67	56,67	14,45	0,71446	0,55	-1,49	1,06
Width	17	31,86	21,67	50,83	11,82	0,69021	0,55	-1,53	1,06
CI	17	0,95	0,77	1,04	0,07	-1,44644	0,55	1,87	1,06

Table 30. Descriptive statistics for length and width of *Glomus* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	48	74,62	25,00	166,67	27,70	1,159	0,34	1,75	0,67
Width	48	66,27	6,00	143,33	27,87	0,73	0,34	0,52	0,67
CI	48	0,89	0,09	1,43	0,17	-1,54	0,34	12,83	0,67

Table 31. Descriptive statistics for length and width of *Glomus* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	17	53,09	33,33	78,33	16,01	0,37	0,55	-1,66	1,06
Width	17	48,63	31,67	71,67	13,77	0,54	0,55	-1,33	1,06
CI	17	0,92	0,79	1,00	0,07	-0,60	0,55	-0,82	1,06

Table 32. Descriptive statistics for length and width of *Endogone* spores (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	13	47,44	21,67	73,33	17,88	-0,10	0,62	-1,44	1,19
Width	13	40,96	20,83	68,33	14,10	0,065	0,62	-0,26	1,19
CI	13	0,88	0,71	1,20	0,13	1,27	0,62	2,72	1,19

Table 33. Descriptive statistics for length and width of *Endogone* spores found in summer (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	9	56,76	36,67	73,33	12,28	-0,40	0,72	-0,81	1,40
Width	9	47,59	33,33	68,33	10,24	0,75	0,72	1,23	1,40
CI	9	0,84	0,71	0,93	0,07	-0,59	0,72	0,02	1,40

Table 34. Descriptive statistics for length and width of *Endogone* spores found in autumn (column designations see Table 1).

Dimension	N	Mean	Min	Max	Std. D.	Skewness	Std. Er Skewness	Kurtosis	Std. Er. Kurtosis
Length	4	26,46	21,67	33,33	5,46	0,65	1,01	-2,17	2,62
Width	4	26,04	20,83	40,00	9,31	1,99	1,01	3,97	2,62
CI	4	0,97	0,77	1,20	0,18	0,31	1,01	0,74	2,62